TREASURY DEPARTMENT
UNITED STATES PUBLIC HEALTH SERVICE
HUGH S. CUMMING, SURGEON GENERAL

# TUBERCULIN

## A REPORT OF A CONFERENCE ON ITS STANDARDIZATION

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## A REPORT OF A CONFERENCE ON ITS STANDARDIZATION[1]

### INTRODUCTION

In June, 1925, the Surgeon General of the United States Public Health Service received the following letter:

SOCIÉTÉ DES NATIONS. LEAGUE OF NATIONS.

*Health Section*

GENEVA, *May 15, 1925.*

DEAR DOCTOR CUMMING: As you may remember, the health committee has decided to undertake the study of standardization of tuberculin. The permanent standard committee appointed a special commission for this purpose, consisting of Professor Calmette (chairman), Professor Léon Bernard, Doctor Madsen, and Doctor Tsurmi.

This commission has come to the conclusion that before submitting proposals concerning the standardization of methods, it will be necessary to obtain as complete information as possible on the methods of preparation and titration in use at the various institutes producing tuberculin.

For the purpose of securing this information from the United States, I am sending you under separate cover 10 questionnaires and explanatory letters prepared by the commission. I should be grateful if you would kindly transmit these forms to the producers of tuberculin in your country.

I am sure that that the commission will appreciate your assistance in this investigation.

With kind regards,

Yours very sincerely,

LUDWICH RAJCHMAN.

Surgeon General CUMMING, H. S.,
*United States Public Health Service,*
*Washington, D. C.*[2]

In July the Director of the Hygienic Laboratory transmitted this questionnaire with a series of additional questions to manufacturers of tuberculin in the United States. The answers were returned, in some instances so slowly, that it was not possible to correlate the information obtained until October of 1925.

On October 5, 1925, after consulting with the officers of the Bureau of Animal Industry and the Research Committee of the National Tuberculosis Association, the Surgeon General sent out the following letter:

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[1] From the Hygienic Laboratory, United States Public Health Service.

[2] The explanatory letter and questionnare mentioned in this letter will be found as Appendices A and B to this report.

TREASURY DEPARTMENT,
BUREAU OF THE PUBLIC HEALTH SERVICE,
OFFICE OF THE SURGEON GENERAL,
*Washington, October 5, 1925.*

DEAR ———: The Public Health Service of the Treasury Department and the Bureau of Animal Industry of the Department of Agriculture, the official agencies concerned in the control of tuberculosis, and the National Tuberculosis Association, which also has a considerable interest in the subject, have carried on investigations for a number of years with the object of endeavoring to standardize the commercial preparations of tuberculin in the market. No very conclusive results have been attained, and the whole subject is in a distinctly unsatisfactory state.

That this is not peculiar to America is evident by the fact that at its last meeting the Health Section of the League of Nations designated a special committee on the standardization of tuberculin under the chairmanship of Professor Calmette of the Pasteur Institute, Paris. This committee has been collecting data wherever they were available, including the United States.

With the view of bringing to bear on this subject the views of a group well qualified to judge of the merits of the problems presented, and with the view of arriving at conclusions which may accompany the American report to the committee of the League of Nations, it has been decided to call a conference for the purpose of discussing the subject, and, if possible, of arriving at conclusions.

The following men have been invited to attend the conference:

Dr. Theobald Smith, president of the International Union against Tuberculosis and president of the National Tuberculosis Association.
Dr. Ludvig Hektoen, past chairman of the Medical Division of the National Research Council.
Dr. Reid Hunt, professor of pharmacology, Harvard University Medical School.
Dr. Marion Dorset, chief of bio-chemic division of the Bureau of Animal Industry.
Dr. George W. McCoy, Director of the Hygienic Laboratory, United States Public Health Service.
And the Research Committee of the National Tuberculosis Association.

The material from which the conclusions will be drawn will be presented in the following way:

1. The present status of the manufacture and use of veterinary tuberculin in America. Dr. E. C. Schroeder, Director, Animal Experiment Station.
2. A discussion of the present methods proposed for standardization of tuberculin. Dr. Esmond R. Long, assistant professor pathology, University of Chicago.
3. The variations in these proposed standards apparent from the use of various pure substances used for tests. Dr. Max Pinner, director serological Division of the Chicago Municipal Tuberculosis Sanatarium.
4. The principles of bacteriological chemical analysis: The application of these principles to commercial large scale production applied to the tubercle bacillus. Dr. Treat B. Johnson, professor of chemistry, Yale University.

The meeting of the council will be held at the Butler Building, 3 B Street SE., Washington, D. C., on October 16, 1925, at 10 a. m. The meeting will be opened by the Surgeon General of the United States Public Health Service and Dr. John R. Mohler, Chief of the Bureau of Animal Industry. The results of the answers to the questionnaires sent out by the League of Nations will be presented by the Hygienic Laboratory.

This meeting is called by the United States Public Health Service, the Bureau of Animal Industry, and the National Tuberculosis Association. It is hoped that you will be able to attend the meeting. The meeting is not open to the public and is confined to the participants mentioned in this letter.

H. S. CUMMING,
*Surgeon General, United States Public Health Service.*
JOHN R. MOHLER,
*Chief, Bureau of Animal Industry.*
LINSLY R. WILLIAMS,
*Managing Director, National Tuberculosis Association.*

This letter called together, on October 16, 1925, a conference to discuss, for America, the question of the standardization of tuberculin, and to obtain, if possible, definite conclusions which would guide American action in this matter and serve as a guide to the Health Section of the League of Nations in its discussion with regard to the American position.

## THE MEMBERS OF THE CONFERENCE

At the conference, representing the United States Public Health Service, the Bureau of Animal Industry, and the National Tuberculosis Association, were Surgeon General H. S. Cumming, of the United States Public Health Service (in the chair); Dr. John R. Mohler, Director of the Bureau of Animal Industry of the Department of Agriculture; Dr. A. M. Stimson, Assistant Surgeon General of the United States Public Health Service; and Dr. Linsly R. Williams, managing director of the National Tuberculosis Association.

The committee serving as a court of decision was composed of Dr. Theobald Smith, president of the National Tuberculosis Association; Dr. George W. McCoy, Director of the Hygenic Labratory; Dr. Reid Hunt, professor of pharmacology, Harvard University Medical School; Dr. Ludvig Hektoen, past chairman of the medical division of the National Research Council; and Dr. Marion Dorset, chief of the biochemic division of the Bureau of Animal Industry.

The Medical Research Committee of the National Tuberculosis Association was represented by Dr. William Charles White (chairman), United States Public Health Service; Dr. Allen K. Krause, Johns Hopkins Hospital, Baltimore; and Dr. Paul A. Lewis, Rockefeller Institute, Princeton, N. J.

The active research work on which to base the discussion was presented by Dr. E. C. Shroeder, of the Bureau of Animal Industry Experiment Station; Dr. Esmond R. Long, department of pathology of the University of Chicago; Dr. Max Pinner, of the Chicago Municipal Tuberculosis Sanatarium; and Dr. Treat B. Johnson, professor of chemistry, Yale University.

## CONCLUSIONS

The conclusions, which follow in answer to three questions, were based upon the subsequent papers and discussion:

QUESTION. Have we sufficient knowledge at the present time to warrant the establishment of a standard for this substance?

ANSWER. We have not at present sufficient information to recommend definite changes in the standardization and testing of tuberculin as now practiced by different countries, nor shall we have until the different methods proposed have been exhaustively studied comparatively.

QUESTION. Can we, with our present knowledge, make important improvements in the present methods of manufacture of tuberculin and what should these improvements be?

ANSWER. The commission believes that a test based upon the following definition will aid in bringing about a uniformity in practice:

Tuberculin O. T. should be defined as a product derived from bouillon cultures of the tubercle bacillus (human type) by filtration and concentration and should have the following essential characteristics:

(*a*) It should cause typical symptoms of allergy (constitutional or skin reactions) in tuberculous animals and at the same time be without effect on normal animals.

(*b*) Its potency should be sufficient to cause the death of tuberculous guinea pigs within 24 hours after intraperitoneal injection of 0.25 gram doses per 500 grams of guinea pig weight.

In practice the strength of other forms of tuberculin should be computed in terms of O. T.

Tuberculin from bovine strains of tubercle bacilli should conform to the standard for O. T. (Old Tuberculin).

Tuberculins from avian strains of tubercle bacillus do not conform to this standard for O. T. and should be considered independently.

It is desirable for Government bureaus concerned with licensing tuberculin to issue a statement of a method with which a product of suitable potency and purity is more or less uniformly obtainable.

QUESTION. What lines of study are the best for us to pursue to enable us to arrive at a better standard of tuberculin?

ANSWER. The commission unanimously approves the present cooperative plan of research which is being carried on by the Research Committee of the National Tuberculosis Association in cooperation with the Hygienic Laboratory of the United States Public Health Service and the Bureau of Animal Industry as the most likely procedure to bring about a better understanding of tuberculin and its action and to enable us to arrive at better methods of use and standardization, and the commission urges that this work be continued.

## SCIENTIFIC PAPERS

Surgeon General CUMMING (calling the meeting to order). It is a great pleasure for me to welcome the members of this conference on the standardization of tuberculin. Every member of it was carefully chosen to represent some broad phase of the biological problem presented by this diagnostic and therapeutic agent—tuberculin.

Study in America has progressed very rapidly both in the Bureau of Animal Industry and through the researches being carried out by the Research Committee of the National Tuberculosis Association, but it is doubtful whether this conference would have been called at this time had it not been for the urge given it by a questionnaire sent out to all countries, including the United States, by the Health Section of the League of Nations looking toward some action in an endeavor to standardize tuberculin.

As you all know we have no official relation with the League of Nations, but so many interests, both human and industrial, are involved in fixing an international standard of this sort that it seems wise to attempt to formulate an American opinion on the subject both for our own guidance and for presentation to the Health Section of the League.

The biologic law [3] in the United States places under our jurisdiction the licensing of all serums, viruses, and toxins which have to do with the prevention and cure of disease in man. With regard to tuberculin this is a very small matter compared with the enormous use of this substance in the animal industry, for which the Bureau of Animal Industry of the Department of Agriculture is responsible. In order to bring together for your consideration the most recent work on the subject, the Bureau of Animal Industry and the Public Health Service have united in asking the Research Committee of the National Tuberculosis Association to join them, and these three bodies are represented in the material which will be presented to you.

Doctor Mohler, who will welcome you, following me, will present the facts which are represented by the work of the Bureau of Animal Industry, with some suggestions as to the benefits which may result from this conference.

There is before you a synopsis [4] of the answers received in connection with the accompanying questionnaire sent out by the Health Section of the League of Nations. It would seem quite within the range of possibility that the conclusions reached here may fittingly

[3] Regulations for the Sale of Viruses, Serums, Toxins, Etc. Miscel. Pub. No. 10, United States Public Health Service. Government Printing Office, Washington, 1923.

[4] This synopsis will be found as Appendix C to this report.

answer these questions, which would be of great service to all who have to deal with this problem:

1. Have we sufficient knowledge at the present time to warrant the establishment of a standard for this substance?

2. Can we, with our present knowledge, make important improvements in the present methods of manufacture of tuberculin, and what should these improvements be?

3. What lines of study are the best for us to pursue to enable us to arrive at a better standard of tuberculin?

What an enormous factor tuberculin plays in the animal industry will be apparent to you from Doctor Mohler's remarks. I would like, in closing this welcome, to comment upon the cooperation here represented of the two great government bodies dealing with this question and the voluntary organization, the National Tuberculosis Association. The cooperation of these bodies should soon result in a completion of our knowledge of this question; and it may be that, if your conclusions to-day outline a feasible program, you may, yourselves, have the opportunity of listening to the finish of the program and the settling of this question in America.

Doctor Houck, the Assistant Director of the Bureau of Animal Industry, tells me that Doctor Mohler can not be present, but that he will present Doctor Mohler's remarks. Doctor Houck.

### Tuberculosis of Livestock

By U. G. Houck, Assistant Director, Bureau of Animal Industry

I regret that Doctor Mohler can not be here himself, but he was suddenly called out of town on a court summons and requested me to present his remarks. This I shall do, and I have taken the liberty of adding a few of my own.

The eradication of tuberculosis of livestock in the United States is a feasible, though huge, undertaking. This work is important from an economic standpoint and also because a certain percentage of human tuberculosis, especially among children, is due to infectious dairy products obtained from tuberculous cows. Much progress already has been made through cooperative Federal and State efforts. Experience has shown that the "area plan" is the most economical method of eradicating bovine tuberculosis. In carrying out this plan all cattle in a county or other prescribed area are tested during an intensive, systematic drive. The "accredited herd method" is favored next from the point of economy and effectiveness. It has been observed that when this disease is eradicated from the cattle in a community it disappears or is greatly reduced among swine. Fowls also are susceptible, and eradication of tuberculosis from our poultry flocks is now receiving consideration.

Over 7,000,000 cattle in 607,000 herds were tuberculin tested in the United States during the last year. The total number of reactors was 214,491 cattle, or about 3 per cent. In other words, tuberculosis was found in the proportion of one-third of an animal in every herd tested. It is self-evident that it is more economical to sacrifice one-third of an animal per herd now than to allow the disease to spread until 50 per cent or more of the animals per herd become tuberculous, as is the case in some European countries. If by taking one-third of an animal now from every herd in this country tuberculosis will be eradicated, the expense will not bankrupt the livestock industry nor will it be too much for the State and National Governments to bear.

There are now 72,283 herds, containing 1,275,063 cattle, in the United States officially accredited as free from tuberculosis, an increase over last year of over 24,000 herds containing more than 354,000 animals. This is an increase of 49.9 per cent in accredited herds during the past year.

There are 921,758 herds, containing over 8,000,000 animals, that have passed one clean test. This is an increase over last year of 392,000 herds, containing more than 3,000,000 cattle.

At the present time there are under direct supervision over 1,000,000 herds of cattle, containing more than 11,000,000 animals, and we have a waiting list of owners of 403,000 herds, containing 3,500,000 cattle, who have voluntarily requested the cooperating forces to test their animals.

In the fiscal year 1924 there were 38 counties placed in a "modified accredited area" status, and in 1925 additional counties were similarly placed to the number of 51, making a total of 89 modified accredited counties. There are in the process of being placed in modified accredited area status a total of 591 counties, which is an increase of 273, or 46 per cent, over last year.

A total of 726 veterinarians are now employed in the tuberculosis eradication work as compared with 565 a year ago This force is divided as follows: Bureau of Animal Industry, 219; States, 246; counties, 261.

The various State legislatures that met during the past winter increased the total State appropriations available for the payment of indemnities from $6,000,000 to more than $11,000,000. It is expected, therefore, that there will be a considerable increase in the total number of cattle tested during the period provided for by most of the legislatures. It is further expected that the States will assume the responsibility of conducting the campaign in each Commonwealth, so that the Federal Government will thereby be relieved of the responsibility of controlling and eradicating tuberculosis of livestock in each State. This is a duty that manifestly belongs to the officials

and livestock owners of each State. It is fit and proper that the Federal Government should act in an advisory way and help to work out the problem for the States; but until each State has an organization, feels the responsibility, and performs the work of controlling the disease we shall fall far short of accomplishing the ideal results. The peak of tuberculosis eradication work will be reached within the next few years, after which there should be a gradual decline in Federal appropriations and the responsibilities that are now shouldered by the department.

The data given shows the magnitude of our field operations, which are based on tuberculin as the diagnostic agent. The Bureau of Animal Industry is the largest producer of tuberculin in the United States. In 1922 it produced 2,977,800 cubic centimeters to carry on these field operations; in 1923 the production was 2,639,750 cubic centimeters; and in 1924 it was 3,121,600 cubic centimeters, which is about five times the combined output of all other producers. And it is expected that the bureau's production will be increased considerably during the current year and probably for years to come.

In addition to the biologic law to which Surgeon General Cumming alluded, the virus-serum-toxin law, approved on March 4, 1913, is in operation in this country. The Department of Agriculture is charged with the responsibility of administering this law. It gives protection to producers and also to those who use tuberculin in the field of animal industry.

Producing establishments engaged in interstate trade operate under licenses granted and regulations and a system of inspection prescribed by the Secretary of Agriculture. It is needless to enter into the details of the virus-serum-toxin law, as no doubt you are familiar with the purposes and provisions which extend even to include imported virus, serum, toxin, and analogous products intended for use in the treatment of domestic animals.

In discussing the subject of fixing an international standard for tuberculin we should keep in mind the study and research that have been completed and what we really know on the subject. The bureau is principally interested from an economic standpoint; and in view of our present knowledge of tuberculosis and tuberculin it seems that our extensive laboratory and field operations are being conducted on a practical, effectual working basis.

There seems to be no doubt that a free discussion of the three questions presented by the Surgeon General in his address will lead to a fitting conclusion.

Surgeon General CUMMING. Doctor Schroeder will now present his paper on "The Present Status of the Manufacture of Veterinary Tuberculin."

## The Present Status of the Manufacture of Veterinary Tuberculin

By E. C. SCHROEDER, Director Animal Experiment Station

In accordance with where and by whom it is made, tuberculin for veterinary use in the United States may be classed under three headings, Federal, State, and commercial.

The Federal, commonly spoken of as Bureau of Animal Industry tuberculin, is made in the biochemic division of the Federal Bureau of Animal Industry, and is estimated to be about 90 per cent of the total amount. I have been informed that a million or more doses are made and distributed monthly. As Dr. Marion Dorset, chief of the biochemic division, under whose direction and supervision this tuberculin is made, is present at this conference, I will leave it to him to give, if necessary, a more detailed account of its preparation and distribution.

State tuberculin is prepared in the laboratories of State livestock institutions in a number of States. It is used exclusively within the States of its origin, and, therefore, the Federal Government has no authority to supervise its production, purity, and potency, and little definite information regarding it is available. Occasionally, samples are sent to the Bureau of Animal Industry at Washington to be tested, in most instances after some question has arisen about their quality, and the tests made with such samples prove that State tuberculin is not invariably as good as it should be. For example, very recently a sample was received which was claimed to have caused reactions in an exceptionally large number of cattle in which no lesions of tuberculosis were found on post-mortem examination. This specimen had a faintly clouded appearance, and with it an abundant sediment was obtained in centrifuge tubes. Microscopic examination of the sediment revealed a large number of presumably dead, acid-fast bacteria and an enormous number of micrococci. It is hardly necessary to say that a tuberculin heavily contaminated with extraneous bacteria may cause reactions resembling tuberculin reactions in animals wholly free from tuberculosis.

All States in the United States do not produce tuberculin for veterinary use, and the methods of production in different States are not identical.

Commercial tuberculin for veterinary use is made in the United States under license issued by the Bureau of Animal Industry by 12 biochemical concerns. The total amount produced last year (1924) was 2,036,854 doses, divided as follows: For intradermic use, 1,617,122 doses; for subcutaneous use, 316,456; liquid for ophthalmic use, 80,137; and ophthalmic disks, 47,559.

As nearly as it is possible to estimate, the above-enumerated doses amount to from 225,000 to 250,000 grams of Koch's Old Tuberculin.

Each concern licensed by the Bureau of Animal Industry has on file in the virus-serum division of the bureau a statement regarding the preparation of its tuberculin. A study of these statements shows that commercial tuberculin is marketed under several different names and forms, as follows:

*Subcutaneous tuberculin*—in most cases a diluted tuberculin, of which each cubic centimeter contains 0.125 gram of Koch's Old Tuberculin. The Bureau of Animal Industry requires that each subcutaneous dose of tuberculin for a bovine animal of average size must contain the equivalent of 0.5 gram of Koch's Old Tuberculin.

*Intradermic tuberculin*—the same as subcutaneous with the exception that it is doubly as strong—that is, each cubic centimeter contains 0.25 gram of Koch's Old Tuberculin.

Purified intradermic tuberculin—an alcoholic precipitate of Koch's Old Tuberculin dissolved in physiological salt solution.

*Ophthalmic liquid*—practically the same as the purified intradermic tuberculin.

*Ophthalmic disks*—alcoholic precipitate of Koch's Old Tuberculin washed, dried, ground, mixed with milk sugar, boric acid, and bicarbonate of soda, and pressed into disks.

*Retest tuberculin*—same as subcutaneous with the exception that each cubic centimeter contains 0.375 in the place of 0.125 gram of Koch's Old Tuberculin.

The statements show many variations in the methods of preparation.

One concern depends entirely on a single, human strain of the tubercle bacillus; another on a single, bovine strain; others use two or more strains, usually a mixture of human and bovine; one uses as many as 7, and one as many as 10 strains.

The medium in which the bacilli are grown varies greatly, both in composition and initial reaction. Veal bouillon, beef bouillon, bouillon made with meat extract, etc., are used, and the glycerin varies from 3 to 7 per cent.

The length of time the cultures are incubated varies, as does also the relation between the volume of medium and its surface area. The temperature at which the tubercle bacilli are killed varies not only in degrees but also the duration of exposure. In most cases Arnold steam sterilizers are used. In one case sterilization in an autoclave under steam pressure is practiced, and in one discontinued or interrupted exposure to heat.

The methods of filtration, precipitation when it is used, the character of the flasks in which the cultures are grown, the methods of testing purity and potency, the preservatives added, etc., all show variations.

Taking this failure of more precise agreement in methods of production among different commercial concerns into account, and the fact that actual tests of commercial tuberculin show that most of it is satisfactory for veterinary purposes, leaves no doubt that tuberculin is a substance which can be subjected to much harsh treatment and many trying vicissitudes without losing its virtue as a diagnostic agent for tuberculosis.

It now remains to say a few words about the importance of veterinary tuberculin.

A report for September, 1925, which gives a summary of the bovine tuberculosis eradication work of the Federal Government in cooperation with various States, shows that 1,231,692 herds, which number 12,302,200 cattle, are now under supervision. These large numbers are constantly increasing, and for some time to come most of the cattle they represent should be tested with tuberculin once per annum.

The bovine tuberculosis eradication project in the United States is progressing along two lines, viz, accredited herds and accredited areas. An accredited herd is one in which three successive semiannual or two successive annual tuberculin tests have been made without a reaction. An accreditation certificate is good for one year only, and its periodic renewal is made dependent upon an annual tuberculin test of the accredited herd without reaction.

An accredited area is one in which, as determined by the tuberculin test, not more than one-half of 1 per cent of the cattle are affected with tuberculosis. At what intervals of time the cattle in such areas are subjected to the tuberculin test I am not quite sure; I believe there is a difference in different localities.

As the bovine tuberculosis eradication work progresses, we will have a constantly increasing number of cattle in the United States that must be tested periodically. The number tested under the supervision of Federal inspectors and State officials during last September (1925) was 665,627, among which 25,198 reacted. During last August (1925) the number was 676,856, with 19,066 reactions.

While the proportion of cattle affected with tuberculosis varies greatly in different States, it is now estimated that approximately 3 per cent, probably a little less, of the total cattle of the United States are tuberculous. Compared with the countries of western Europe, in some of which the proportion of tuberculous cattle is from 10 to 15 times greater, this is a very favorable condition. But, nevertheless, with the tuberculin testing now in progress in the United States, it means the annual condemnation and slaughter, on the basis of what the tests reveals, of from 225,000 to 250,000 head of cattle, the value of which, estimated by using the average per head value of the

cattle on American farms, given in the last Yearbook (1924) of the United States Department of Agriculture, is between $8,000,000 and the $9,000,000.

The tuberculin testing is being done by Federal and State inspectors and so-called accredited veterinarians. The latter are veterinary practitioners who have supplied evidence that they are qualified and can be trusted to make tuberculin tests.

It is the State inspectors and accredited veterinarians through whom, I assume, the State and commercial tuberculin comes to use; and as this means that State and commercial tuberculin is an agent used to test several million cattle annually, no doubt can be entertained that it is urgently necessary that it should be pure and potent.

In cattle testing we must aim first of all to insure that the tuberculin used is amply potent. A standard potency, no doubt desirable, has not been proved to be essential, since cattle that are free from tuberculosis do not react even when they receive subcutaneous or intravenous injections of 8 to 10 times the quantity of tuberculin required to cause a reaction in a tuberculous animal, and are in no discoverable way harmed by such large doses. The harm suffered by tuberculous cattle from excessive doses has not proved serious enough to give it economic importance, in part because a tuberculin reaction is equivalent to a condemnation which terminates the usefulness of the animal.

The expression "doses of tuberculin" may be misleading when the number of doses distributed for veterinary use is compared with the available records of the number of animals tested. The discrepancy in this instance is explained by the fact that a very large proportion of the tested animals are subjected simultaneously to two or more tests—as, for example, the intradermic and the subcutaneous; the intradermic and a test somewhat similar to the intradermic in which the tuberculin is injected into the vulval lips at the junction of the mucosa and skin; the intradermic and the ophthalmic; etc.; and in making the ophthalmic test it is a common practice to introduce into the conjunctival sac first what is designated as a sensitizing and later a test dose of tuberculin. The intradermic test, made by injecting tuberculin into the delicate skin of the tail, is the test most commonly used in the United States.

In addition to the importance veterinary tuberculin derives in the United States from its extensive use, it has a further importance due to the fact that no method of controlling or eradicating tuberculosis among the lower animals has been devised which can hope to be successful without its use at some stage. Tuberculous cattle often become dangerous disseminators of tubercle bacilli long before their health is determinably impaired or recognizable symptoms of disease

have developed; and even when other methods than tuberculin testing and the slaughter of reacting cattle are used, such as the Bang method of feeding calves pasteurized milk, or methods which depend upon physical examinations, milk examinations, etc., we must eventually turn to tuberculin to measure the progress that is being made and to distinguish with reasonable certainty between safe and dangerous animals.

A few words on the reliability of the tuberculin test as applied to cattle may not be amiss.

As practically all cattle that react with tuberculin in the United States are killed and examined post mortem, we have millions of animals from which we can determine the reliability of the tuberculin test as far as this depends upon the presence of tuberculous lesions in animals that react. Very recently I had occasion to study the data, and found that something less than 1 reacting animal among every 400 tested can not be proved to be tuberculous by the discovery of microscopic lesions of the disease in its body. Such so-called "no-lesion tuberculin reacting animals," in about one case out of every four, by searching microscopic examinations and guinea-pig inoculations, have been proved to be actually infected with tubercle bacilli—that is to say, have been proved to be early cases of tuberculosis. Hence the reliability of the tuberculin test, as far as it is affected by reacting animals in which no lesions of tuberculosis can be discovered on autopsy, is better than 99.75 per cent.

To what extent the tuberculin test is unreliable because of the failure of tuberculous animals to react with it is a more difficult question to answer, as the number of animals which do not react and are killed early enough after the test to exclude errors due to subsequent infection is very small.

At the experiment station of the Bureau of Animal Industry, where all cattle are currently tested with tuberculin and eventually subjected to fairly careful post-mortem examinations, we have practically no records of animals, never previously exposed to tuberculin, which failed to react and were afterwards found, without subsequent, definitely known or intentional exposure to infection, to be affected with tuberculosis. In other words, if an animal fails to react to tuberculin the first time it is tested, the experience of the bureau's experiment station strongly indicates that it is certainly free from tuberculosis. The station's experience in this respect extends over a period of more than a quarter of a century and concerns several thousand head of cattle.

Failure of tuberculous animals to react when the tuberculin test is applied a second, third, or fourth time is another matter. There are tuberculous animals which react once and never again, and the sta-

tion has had a number of cattle under observation which reacted with two, three, or four semiannual tests and thereafter were so tolerant for tuberculin that large doses injected subcutaneously, intra-abdominally, or intravenously caused no perceptible reaction.

This failure of a tuberculous animal to react with tuberculin must not be mistaken as evidence that the disease has been arrested. A number of such animals kept under observation to determine what the course of the disease would be, without ever regaining their sensitiveness for tuberculin, eventually died of tuberculosis, and nothing was observed in the course of the disease or during the autopsy at its termination to offer an explanation for the lack of tuberculin sensitiveness.

If the statements made by some of our bovine tuberculosis eradication inspectors are reliable, it is amazing how small the dose that deprives a tuberculosis animal of its tuberculin sensitiveness is in some instances. I have been told that some tuberculous cattle lose their sensitiveness after a single intradermic dose.

Of course it is well known that occasionally a far advanced, usually physically evident, case of tuberculosis among cattle is wholly lacking in tuberculin sensitiveness.

The greatest weakness of tuberculin is that it gives us no clew to the age, extent, character, or location of the tuberculous lesions in the bodies of reacting animals, and the fact that tuberculous animals, after one or more exposures to tuberculin, may wholly lose their tuberculin sensitiveness justifies the slogan, "Once a reactor an animal must thereafter always be looked upon as tuberculous."

I believe that is all I need say on the subject of veterinary tuberculin at the present time.

Surgeon General CUMMING. Doctor Long will give us a "Discussion of the Present Methods Proposed for Standardization of Tuberculin."

### Discussion of the Present Methods Proposed for Standardization of Tuberculin

By ESMOND R. LONG, M. D., Department of Pathology, University of Chicago

I have been asked to discuss some of the principles underlying the standardization of tuberculin, and to say something concerning the large-scale production of tuberculin from synthetic media. We must admit at the outset that we do not know the nature of the active principle of tuberculin. At the present time the only definition we can make of tuberculin is " that substance which elicits what, by common consent, is called the 'tuberculin reaction.' "

The ideal method of measuring the strength of a tuberculin preparation would be a chemical one. Unfortunately at present we are not sufficiently well acquainted with the chemical nature of the active principle to make use of a chemical method for standardization. However, work is under way in a number of laboratories which may

ultimately make such a method possible. At the very least it may be possible to standardize the production of tuberculin chemically in a standard medium of known chemical composition. I understand that Doctor Dorset in the Bureau of Animal Industry uses such a medium to great advantage, and I have devised one on which two large pharmaceutical houses, which are cooperating with the Research Committee of the National Tuberculosis Association, have prepared huge quantities of tuberculin for research purposes.

The important ingredients of this medium are asparagine, ammonium citrate, and glycerol, with acid and alkaline salts present in such amount that the medium is buffered and requires no titration. As the medium contains no protein originally, chemical methods based on the amount of protein occurring in such medium after growth of tubercle bacilli may yield some information as to the potency of such preparations, and the bacilli grown are produced in a standard way, permitting the isolation of important products of uniform character. As will be brought out by Doctor Johnson later, some of these products have high tuberculin activity. In the development of this field there is much prospect for advance toward chemical standardization.

At present, however, in the lack of definite knowledge on the chemical nature of the active principle of tuberculin, we must continue with biological methods of standardization—i. e., methods based on the tuberculin reaction itself. The tuberculin reaction is an allergic reaction, or reaction of hypersensitiveness, and the standardization of tuberculin is based in most laboratories on the hypersensitiveness of the tuberculous guinea pig. Both the general and the local reaction have been used in the evaluation. Recently the antigenic capacity of tuberculin in the precipitin and complement fixation reactions with the serum of tuberculous animals has been proposed as a measure.

The advantages and disadvantages of the methods outlined above may be conveniently analyzed under four headings: (1) The methods based on the lethal dose for the tuberculous guinea pig, (2) the method based on the cutaneous test, (3) the complement-fixation method of Watson and Heath, and (4) the precipitin method of Dreyer and Vollum.

*Methods based on the lethal dose for the tuberculous guinea pig.*—The original Koch standardization, the Frankfurt method, and the method of the United States Bureau of Animal Industry are, to some extent, similar in their advantages and disadvantages.

The Koch method has the advantage of extreme simplicity. A preparation of tuberculin passes the test or fails according as 0.5 cubic centimeter kills or fails to kill a tuberculous guinea pig. Prac-

tically no labor is required, and the test needs only 30 hours for its completion.

The disadvantages, however, are marked. In the first place, the test is too gross. The criticism applies equally to all methods based on the lethal dose for the tuberculous guinea pig. There are too many unknown factors. A positive result, the death of the guinea pig represents the summation of these unknown factors. The first unknown factor is the extent of the tuberculosis itself within the guinea pig. The animal is already absorbing a great or small amount of poisonous material, as the course of the disease present is rapid or slow. The general tuberculin reaction is added to this unknown factor. The general tuberculin reaction includes the summation of the individual tuberculin reactions between sensitized tissue and tuberculin in all parts of the body. Probably all tissues are sensitized to some degree, but the degree for the different tissues is not known. There is evidence that it varies in the different tissues (unpublished observations of the author on the suprarenal, kidney, testis and skin). Perfection in a test based on the strength of the general reaction could not be obtained without a series of animals of the same weight with exactly the same amount of tuberculosis at the time of the test, with livers, spleens, brains, kidneys, etc., of the same size, with the same amount of muscle, the same amount of skin and subcutaneous tissue, etc. This is obviously impossible of attainment. Even when a large series of animals is used, accordingly, and a lethal effect on the majority is considered the basis for a standard, individual variation is so great as to rob the test of much of its value.

There is another weighty objection. The test is quantitative only in a "pass or fail" manner. No unit is established. A unit is very desirable, not only in the diagnostic, but particularly in the therapeutic, use of tuberculin. Two preparations of tuberculin might pass the test, and yet one of them be twice as potent as the other. This opens the possibility of gross error in therapeutic dosage.

The Frankfurt method has the advantage of relative simplicity, and recognizes the variability of animals as respects the lethal dose, without, however, avoiding it. An advance over the Koch procedure is achieved in that an attempt is made to measure the degree by which superstrength preparations surpass the standard, and a basis is set for dilution to a common strength. However, no true unit is established by which the strength of a preparation may be determined without going through the laborious process of comparison with a standard tuberculin on a large series of animals.

The method of the United States Bureau of Animal Industry is an improved lethal dose method in which careful control is exercised with respect to the degree of sensitiveness of the animals used. The actual measurement of tuberculin is made only after extended pre-

liminary tests have led to the selection of animals of suitable and comparable sensitiveness. The method is, however, laborious, and large numbers of animals are required, a great many being killed in the determination of sensitiveness before the test proper is begun.

At the best, even when well controlled and based on averages, methods founded on the lethal dose leave much to be desired, in view of the unquestionable complexity of the general tuberculin reaction. The method of the Bureau of Animal Industry, like the other methods of this type, sets up a required standard which must be met before a tuberculin is acceptable, but it does not establish a unit which can be used in measuring doses.

In favor of the lethal dose methods in general, in contrast to the serum methods discussed below, is the fact that the allergic state of the tuberculous animal is a factor in the measurement. It is the allergic state of the patient or suspected animal which is of significance in the diagnosis and treatment of tuberculosis by tuberculin. There is, therefore, in these methods no such dissociation of standardizing principle and diagnostic and therapeutic action as in the serum standardization tests analyzed below.

*The intracutaneous test of Lewis and Aronson.*—This test, based on the intracutaneous test of Roemer, has the great advantage of close association between the standardizing test and the use to which the product is adapted. The substance is standardized with respect to the skin of an allergic animal and later used on the skin of an allergic patient. Furthermore, it is easy of application, requires no special technique, takes little time, and is economical of animals.

It would perhaps be the most useful method were it not for one serious defect. The reactive capacity of the skin varies tremendously in a series of tuberculous guinea pigs, even when these are all injected at the same time. Numerous authors have stressed this point, and Lewis and Aronson themselves are careful to state on the basis of their own protocols: "It is evident that the individual variation in reaction of the animals is wide." Naturally the value of a test in which the strength of reaction is an important measuring factor is much reduced by such variability.

To the present methods based on the allergic response in the tuberculous guinea pig I have added one in which the hyper-sensitiveness of the germinal cells of the tuberculous animal is used as the measuring rod for tuberculin activity. This type of tuberculin reaction I have described in the literature as the "testicle-tuberculin reaction," and more recently as the "spermatocyte reaction." The high degree of susceptibility of the germ cells of the tuberculous guinea pig to the action of tuberculin makes the testicle-tuberculin reaction one of great delicacy. The reaction is specific for tuberculin. Other substance do not elicit a tuberculin type of reaction in the testical of the

tuberculous guinea pig, and tuberculin does not cause any reaction in the testical of the normal guinea pig. On the basis of the change observed microscopically in the testicle of the tuberculous guinea pig which has been injected with tuberculin, the strength of a tuberculin preparation may be evaluated.

This method defines a "spermatocyte unit" of tuberculin as that quantity just sufficient to abolish spermatogenesis on injection in a volume of 0.1 cubic centimeter into the testicle of 400-gram guinea pig with a mild, localized tuberculosis of one month's duration.

Potent preparations of Old Tuberculin average about 10,000 units by this method. The method is new and will, of course, have to be submitted to extensive trial before its advantages and disadvantages can be fully determined.

*The complement-fixation method of Watson and Heath.*—This method has the primary advantage that it furnishes a unit in which all doses of tuberculin can be measured. It has another great advantage in that a standard reagent, the antiserum, can be prepared in a quantity sufficient for a great number of tests. The antiserum appears to stand aging moderately well, and thus furnishes a reagent of somewhat greater reacting constancy than the tuberculous guinea pig. It is time saving in that the test may be carried out at once on the receipt of the material to be tested. No delay for the sensitization of guinea pigs is required.

On the other hand, it suffers from the great defect of complete dissociation of standardizing test and the use to which the material is adapted. This is no evidence that the tuberculin reaction and the antibodies which can be detected in the serum are even related. In fact Calmette,[5] on the basis of long study, states that purified tuberculin will not act as antigen in the serum test. If this is true, the extraordinarily close agreement obtained by Watson and Heath, using the complement-fixation method, and Schroeder, using the lethal dose method, is nothing more than a coincidence, and leaves open the possibility that samples of tuberculin might be encountered which would fix complement well in the presence of the antiserum, and yet be low in tuberculin potency. Indeed, if Calmette's contention is correct, a highly purified tuberculin, free from certain extraneous products of the tubercle bacillus which are antigenic to the antiserum, would fail altogether to give the complement-fixation test. Evidence corroborating Calmette's view is cited in discussing the precipitin method of standardization.

*The precipitin method of Dreyer and Vollum.*—Like the fixation method just discussed, the precipitin method has the advantage of establishing a unit in which dosage can be calculated. Similarly, the

[5] L'Infection Bacillaire et la Tuberculose, 1922, p. 576.

essential reagent, the antiserum, is one which can be prepared in large quantities and which is of constant potency for a given lot. It has an advantage over the other serum procedure in that the precipitin method requires fewer fresh reagents and is easier of application.

On the other hand, as in the case of the fixation method, no relation has yet been established between the type of reaction in which the substance is actually used and that used in standardizing the product. In this case also the work of others indicates that the two reactions do not measure the same thing (Calmette[6]). The antiserum is not an antituberculin. It is more than possible that potent tuberculins might be encountered giving a weak precipitin test, and vice versa. In this case also highly purified tuberculins might be expected to give a weaker result than crude tuberculins. The reason for this is the simple fact that the serum prepared is an antiserum not to the active principle of tuberculin alone, but to all the antigenic proteins of the tubercle bacillus. Until there is proof that all of these proteins are potent as tuberculin, the use of such a method of standardizing tuberculin is illogical.

The confusion which has existed concerning the relation of the tuberculin skin test and serum antibody reactions in tuberculosis has been considerably reduced by the recent studies of Zinsser and his colleagues, Julia T. Parker, Wayman, and Mueller. By a method of extraction of tubercle bacilli, precipitation of the extract with acetic acid, and filtration, the investigators have secured clear solutions antigenic in both the skin test and serum antibody reactions with tuberculosis serum. At first they were inclined to believe that the two actions were due to the same active principle; but in a recent report on the continuation of this work, Mueller[7] states. "It has finally been possible to show definitely that they (precipitin reaction with tuberculosis serum and the tuberculin skin test) are dependent upon entirely different substances in the tuberculin." Mueller is of the opinion that the substance active in the precipitin test is a carbohydrate analogous to the gum found by Dochez and Avery to be active as an antigen with antipneumococcus serum, while he thinks the substance active in the tuberculin skin test is chiefly protein.

In conclusion I wish simply to say that in my opinion a biological standardizing method should be based on a type of true tuberculin reaction. The evidence offered in support of the serum methods of standardization is not sufficient to prove that these methods and those based on a true tuberculin reaction are measuring the same thing. Which one of the standardizing methods based on the allergic tuberculin reaction is most suitable is a matter for debate. I hope

[6] Ibid., pp. 573-574.

[7] Proc. Soc. Exper. Biol. and Med., 1925, 22, p. 219.

that some time facilities can be afforded for large-scale comparison of the value of the several methods proposed.

Surgeon General CUMMING. Doctor Pinner will give us "The Relation of Serologic Reactions and Tuberculin Activity in Derivatives of Tubercle Bacilli."

### The Relation of Serologic Reactions and Tuberculin Activity in Derivatives of Tubercle Bacilli[8]

By MAX PINNER

It is the purpose of this report to present evidence from the literature and from our own work to prove that serologic methods, such as precipitation and complement fixation, are unsuitable for the standardization of tuberculin. Besides accuracy, sensitiveness, and specificity, an acceptable method for the standardization of tuberculin must be applicable to any substance derived from tubercule bacilli which has any tuberculin effect on tuberculous animals and not only to Old Tuberculin.

With various tubercle bacilli preparations complement fixation, precipitation, and allergic reactions in tuberculous guinea pigs were performed.

The following methods were used in this work:

1. *Complement fixation* was done with an anti-sheep serum, using two hemolytic units of amboceptor and two units of complement. The anticomplementary titration was done with saline instead of serum; in the antigenic titration pooled serum of at least three tuberculous patients was used, each serum giving a four plus fixation with our routine antigen. Having thus determined the anticomplementary and the antigenic unit of each preparation, the antigenic quotient was determined by dividing the anticomplementary by the antigenic unit. This antigenic quotient is a numerical expression of the so-called range and has proved in a large series of preparations a reliable indicator of the antigenic activity in complement fixation. This empirical finding receives a further support by the fact that in more than 80 preparations tested so far it was found, as a general rule that the antigenic quotient increases with decreasing antigenic unit. This is shown in Table 1.

TABLE 1

| Preparation | Antigenic unit | Antigenic quotient | Preparation | Antigenic unit | Antigenic quotient |
|---|---|---|---|---|---|
| | *Mg.* | | | *Mg.* | |
| $B_1$ | | 1.0 | 311 | 0.025 | 4.0 |
| Wa A | .1 | 1.0 | O | .0085 | 8.0 |
| Tebeprotin | .1 | 1.0 | $E_1$ | .00408 | 5.0 |
| V | .05 | 2.0 | $F_3$ | .003 | 20.0 |
| Wa-alc | .026 | 2.5 | | | |

[8] From the research laboratories of the Municipal Tuberculosis Sanitarium, Chicago, Ill.

Primary incubation was three-fourths hour at 37° C., and second incubation at 37° C. for one-half hour.

2. *Precipitation.*—This method was found to be unreliable in the results and subject to a considerable personal error in recording the readings. An important obstacle in the general application of the precipitation test is the fact that many of the preparations to be tested can not be obtained in clear solutions. Suspensions have to be centrifuged and the supernatant fluid must be used for the test, and this obviously does not contain chemically and biologically the substances identical with the sediment. In the case of alcoholic extracts, dilution with saline solution is necessary, which yields more or less opaque emulsions. It was frequently noticed that dilutions of 1:1,000 produced quantitatively the same precipitate with positive serum as the stock solution. As test serum similarly pooled serum was used as in complement fixation. The test was always performed by layering the antigen upon the serum. Readings were taken 1 and 2 hours after incubation and 24 hours after standing at room temperature.

3. *Allergic reactions.*—For intracutaneous reactions 1 milligram dry weight in a volume of 0.1 cubic centimeter was injected into the skin of tuberculous guinea pigs which had been infected three to four weeks before the test and which received as a control 1 milligram of Old Tuberculin intradermally. The relatively large amount, 1 milligram, was chosen because it was expected that some of the substances tested would elicit very weak reactions or none at all.

On account of lack of time and animals, the killing power for tuberculous guinea pigs was determined only for a few representative substances. The Koch procedure was followed.

As to the different derivatives of tubercle bacilli used in this work, no attempt was made to obtain substances of chemical purity; only a rough division into different groups according to solubility was aimed at. This work is being continued at the present time with the pure substances produced by Doctor Johnson. It was possible to include only three of these preparations in the present report. Table 2 shows the results of our work in tabulated form. The conclusion must be drawn that the activity of tubercle-bacillus preparations as antigens in complement fixation and in precipitation does not parallel their tuberculin activity in the tuberculous animal. As far as these comparative studies have progressed to-day, and I may emphasize that we feel that they are far from completion, it appears that the alcohol-soluble substances of the tubercle bacillus are the most active ones in complement fixation (1) and Doctor Long's work (2) indicates that the tuberculin activity is closely associated with substances of the nature of proteins. No statement can be made as yet as to the chemical nature of the substances involved

in the precipitation phenomenon. Titration for complement-fixing power was done on seven substances, which Doctor Long isolated from synthetic tubercle bacilli broth and which he has tested for their tuberculin activity. In a dose of 0.5 milligram none of these preparations fixed complement with tuberculosis sera. Doctor Long found that in a dose of 0.1 milligram two of these preparations gave a one-plus skin test, four a two-plus, and one a three-plus reaction. Calmette (12) states that the more purified tuberculins are, the less active they are in serologic reactions.

TABLE 2

| Preparation | Nature | Complement fixation | | Precipitation | Skin reaction after the injection of 1 mg. | Lethal for tuberculous guinea pig, of 250 gm. weight (c. c.) | Not lethal for tuberculous guinea pig, of 250 gm. weight (c. c.) |
|---|---|---|---|---|---|---|---|
| | | Antigenic unit (mgr.) | Anti-complementary unit | | | | |
| O. T | Old Tuberculin, P. D. & Co. 0.071122-L. | — | 0.50 | + | 1.5 | 0.2 | 0.1 |
| | Residues after extraction with— | | | | | | |
| V | Acetone, 20 minutes | 0.05 | 2.00 | ++ | 2.0 | | 50 |
| X | Acetone, 5 hours | — | .75 | ++ | 1.5 | | |
| $B_1$ | Acetone, 50 hours | .1 | 1.00 | ++ | 1.0 | | |
| Q | Alcohol, 30 hours | — | .75 | + | 1.0 | | |
| $G_1$ | Alcohol and acetone, 20 hours each. | — | 0 | ++ | 1.2 | | |
| $H_1$ | Acetone and alcohol, 20 hours each. | — | 0 | ++ | 1.0 | | |
| $I_{III}$ | Acetone, alcohol, and ether. | — | .25 | + | 1.3 | | |
| $G_2$ | Acetone, alcohol, ether, and chloroform. | — | .75 | ++ | 1.5 | 50 | 20 |
| Wassermann-antigen | Tetralin | .10 | 1.00 | ++ | 1.0 | | |
| W-A-E | Tetralin and alcohol | | 0 | + | 1.5 | | |
| Dreyer | Formalin and acetone | — | 0 | ++ | .5 | | |
| Tebeprotin | A soluble protein product, N=12.3 per cent. | .10 | 1.00 | + | [1]0 | | |
| 304 | Proteins containing N:14.38 per cent. | — | .50 | + | .8 | | |
| 308 | Proteins containing N:14.99 per cent. | — | .75 | ++ | 1.2 | | 20 |
| 311 | Proteins containing N:14.61 per cent. | .025 | 4.00 | ++ | 1.0 | 10 | 5 |
| | Alcohol extracts after extraction with— | | | | | | |
| $E_1$ | Acetone | .00408 | 5.00 | ++ | .5 | | |
| $F_5$ | do | .003 | 20.00 | + | 0 | | |
| O | Acetone, ether, and chloroform. | .0085 | 8.00 | ++ | 0 | | 750 |
| Wa-a | Tetralin | .026 | 2.50 | + | .3 | | |
| L | Acetone extract after alcohol extraction. | — | 0 | ++ | 1.0 | | |

[1] Only 0.1 mg. injected.

Complement fixation and precipitation do not run parallel in tuberculosis, as shown by statistics based on unpublished work by Sweany and Corper. Although the absolute number of positive and negative complement-fixation and precipitation tests in 473 sera is very nearly the same, an agreement of the two reactions is found only in 40.6 per cent of the cases. (Fig. 1.)

Dreyer and Vollum (3) published in 1924 "A Precipitin Method for the Standardization of Old Tuberculin." Their test serum is an immune serum produced in a horse after the injection of Dreyer's defatted vaccine. It appears objectionable to use for this purpose an arbitrary immune serum against only one particular group of substances. Dienes and his coworkers (4) have shown that the injection of different chemical substances of the tubercle bacillus into animals will produce immune sera which react only with those partial antigens used in immunization. Dreyer and Vollum tested only Old Tuberculin by their method and found errors up to 97 per cent of the standard in comparing the results of precipitation with skin tests on human beings. In a series of experiments I tried to duplicate

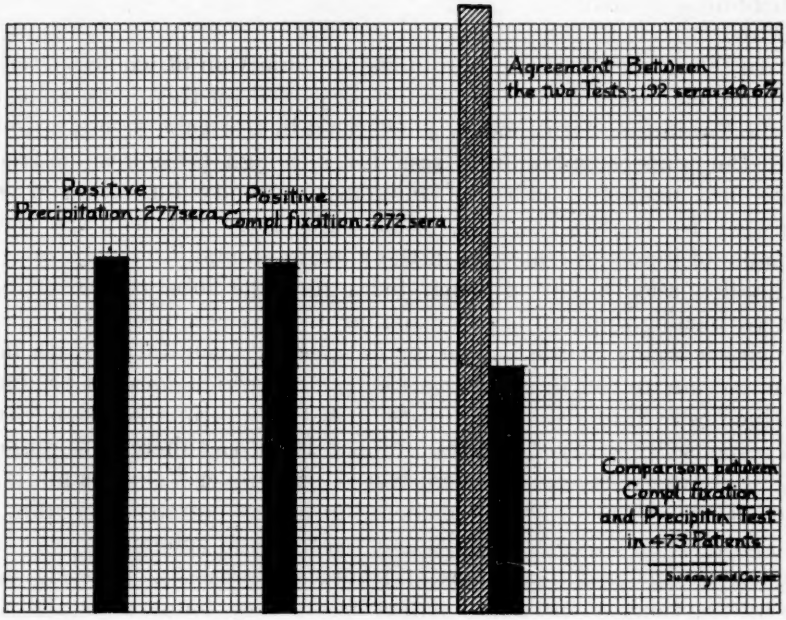


FIG. 1

Dreyer and Vollum's results, following closely their cumbersome technique. Instead of their immune serum, sera from tuberculous patients which gave a four-plus complement fixation were used. It was found that Old Tuberculin frequently does not give any precipitate with such sera, and, when precipitates were obtained, their differences in strength with various tuberculin dilutions were never sufficiently clean cut to allow of a quantitative comparison.

The objections against Dreyer and Vollum's precipitin method may be summarized as follows: An arbitrary immune serum against defatted tubercle bacilli is not acceptable as a standard test object. The method seems to yield uncertain results as applied to Old Tuberculin. The method yields absolutely incorrect results when applied to

other bacillary products than Old Tuberculin. The method is not universally applicable to any tubercle-bacillus derivative which may elicit allergic reactions in the tuberculous animals. These statements are further supported by the work of Zinsser and his associates and by Laidlow and Dudley, who separated a fraction from tubercle bacilli which gives specific precipitation with tuberculosis serum but does not elicit any allergic reactions in tuberculous animals. Zinsser and Mueller (5) arrived at the conclusion "that the bacterial extracts representing the antigens for these reactions (sc. lic. allergy and precipitation) are separable, the residue material being particularly concerned in the specific precipitations with immune serum, the so-called nucleoprotein being associated with allergic reactions in the tuberculous animals."

Also:

"In the case of bacterial immunization, injection of whole bacteria and the bacterial extracts as usually employed for this purpose leads to the formation of antibodies which react in test-tube precipitations with the nonprotein bacterial residues, although these residue fractions are neither capable of inducing antibody formation when injected by themselves, nor, when sufficiently purified, are they capable of inducing reactions in the allergic animal."

Laidlow and Dudley (6) separated a gum-like substance from tubercle bacilli, apparently very similar to Zinsser's residue antigen, which according to the author, gives specific precipitation with immune serum up to a dilution 1: 6,400,000, but which does not produce antibodies in animals. They state: "A fairly strong solution gave no response when injected intracutaneously into a tuberculous guinea pig." If these substances were tested by Dreyer-Vollum's method they would erroneously be considered as highly potent tuberculin.

The relation between complement fixation and tuberculin activity has been discussed by Dienes and his associates (7). Testing different fractions of tubercle bacillus broth, they found a parallelism between the antigenic activity of their fraction in the complement fixation and the minimal dose eliciting a skin reaction in tuberculous guinea pigs. My own work has been done only on fractions of the bacillus itself and not on broth products. A numeric comparison of Dienes results shows that, although there exists in general a certain parallelism between complement binding power and allergic reactions in their preparations, it is not strict and not, by far, sufficiently accurate to measure the allergic activity by complement fixation. In previous work Dienes and his coworkers (4.8) have emphasized the high potency of alcoholic extracts of tubercle bacilli in the complement fixation, quite in agreement with our own work. The parallelism stated for broth products does certainly not extend to these bacillary lipoids.

Watson and Heath (9) advocated complement fixation as a method for the standardization of O. T. They claim that the antigenic activity of O. T. in complement fixation accurately parallels its allergic strength. Their work consisted exclusively in comparing various makes of Old Tuberculin. If such comparative studies are extended to other bacillary preparations, as in the work presented here, a striking divergence between the two activities is found. An indirect proof of the unsuitability of standardizing tuberculin by serologic methods is given by a study by Čepulič (10) from Much's institution. With three different Old Tuberculins standardized by the Frankfurt method, complement fixation tests were carried out on the same patients sera. Some of the representative results are shown in Table 3.

TABLE 3.—*Complement fixation with various Old-Tuberculins—all standardized with the Frankfurt method*

[Čepulič]

| Patient | Old Tuberculin | | |
|---|---|---|---|
| | Höchst | Ruete-Enoch | Sächs Ser-W |
| 1 | ++++ | ++++ | ++++ |
| 2 | ++++ | ++++ | ++ |
| 3 | ++ | ++ | ++++ |
| 4 | 0 | ++++ | ++++ |
| 5 | +++ | 0 | + |
| 6 | 0 | ++ | 0 |
| 7 | 0 | 0 | +++ |

In a recent study on pollen antigens Armstrong (11) arrived at the conclusion that the complement binding and the allergic activity are not quantitatively related.

On the ground of this evidence it may be said that it is not possible to standardize tuberculins by means of the complement-fixation test.

In the complex relation between humoral reactions and allergy in tuberculosis the work of the last two decades has well established the fact that these two phenomena are only very loosely connected. Animals will for example readily respond to injections of various bacillary extracts with the production of serum antibodies, but they will not show any allergic reaction unless tuberculous tissue be formed in them. Some of the recent work reported here shows that serum reactions and allergy—much more so than in the host—are dependent on dissociable substances in the bacillus. This justifies the broad statement that no humoral reaction can measure the allergic activity of a bacillary product.

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Surgeon General CUMMING. Doctor Johnson will give us "The Principles of Bacteriological Chemical Analysis and the Application of These Principles to Commercial Large-Scale Production as Applied to the Tubercle Bacillus."

**The Principles of Bacteriological Chemical Analysis and the Application of These Principles to Commercial Large-Scale Production as Applied to the Tubercle Bacillus**

By TREAT B. JOHNSON, Yale University

In order to lay out a definite plan of analytical technique which will enable the chemist to determine quantitatively the various constituents of a bacterial cell, it is first necessary to know all the characteristic fundamental constituents which function in the structure of the cell. While we have to-day positive evidence of the existence of many of these cell constituents. such as proteins, fats, carbohydrates, etc., it is true that our knowledge of the actual composition of the bacterial cell is far from complete, and consequently we are unable to make an application of an analytical method which will reveal to us the true composition from a quantitative point of view.

At this time it is quite apparent, and agreed by those who are interested in the development of chemical bacteriology, that a study of the chemical constituents of bacterial cells and of the products of cell metabolism are most important; but as long as the condition prevails that we have no thorough and severely tested method of analysis it will be impossible for the chemist to make any advance in this field, and we will remain in that state of chaos and chemical ignorance which prevails at the present time.

An accurate knowledge of cell constitution will never be obtained and the presence of hidden combinations revealed until analytical methods are adopted which are based on an accurate understanding of the distribution of fundamental elements functioning in the constituents of the cell. In order to obtain data which will enable the chemist to establish and adopt a practical analytical technique,

we must follow the distribution in the cell of the three elements—nitrogen, phosphorus, and sulphur. A knowledge of the distribution of nitrogen is absolutely essential as a guide post to reveal hidden combinations containing this element, and in the case of phosphorus we are dealing with an element which is always associated with organic structures characteristic of phosphatides and nucleic acids. According to new biological data which has been presented in recent years, it is very important to follow in bacterial analysis the distribution of sulphur. Oxidation and reduction changes in cell life are undoubtedly influenced by the presence of organic combinations containing this element. Whether sulphur actually functions in the metabolic changes of the tubercle bacillus cell remains to be established.

In order to produce data of any value leading to an accurate knowledge of the composition of cells, both qualitatively and quantitatively, it will be necessary to consider also very carefully several influencing factors, or the data obtained by analysis will be of no practical value and consequently will represent so much wasted time and thought.

In the light of the rapid alterations which cells can undergo, due to the influence of enzymes capable of stimulating autolytic changes, it is necessary that every chemist who undertakes chemical research in this field have some knowledge of the nature of the biological conditions influencing and altering the composition of the cell.

In the first place we must consider carefully the question of conditions influencing the growth of the bacteria. It is very important that the purity of the necessary reagents composing our synthetic media be accurately known and that the media be of uniform composition in all work, otherwise metabolic changes may be introduced in cell growth which will lead to constitutional changes that can not be revealed by any analytical procedure under our control. Again, after final growth and maturity of the cell, it is very important that the bacteria to be studied be carefully separated and washed free from the culture media, so that such contamination will not introduce errors into our analytical results.

Our experience in the Yale laboratories with tubercle bacillus has already taught us that it is very necessary to know under what conditions the death of a cell is brought about before one proceeds to apply his analytical procedure. The data obtained by analysis of a tubercle bacillus cell killed by chemical treatment at temperatures below 37° C. will not be in agreement with data obtained by analysis of cells killed by heating at or above 100° C. Especially is this true if one proposes to determine, for example, the actual content of water-soluble protein. We now know that a water-soluble protein fraction which can be extracted from cells that have not been autoclaved is rendered absolutely insoluble by coagulation if the fresh cells are first heated above 100° C. This is a very important fact and one which must

always be considered in the development of any method to be utilized in manufacturing an active tuberculin preparation from the tubercle bacillus cell.

From what we have been able to learn from our analytical experience with desiccated tubercle bacilli it is apparently necessary to free cells from fat before proceeding with the application of later analytical procedures. Here again we are dealing with a technique which must be carefully controlled or alterations in composition will be introduced which will decidedly affect our analytical results. In those cases where we are dealing with a mixture of fatty acids, fats, and waxes spread throughout the structure of the cell, it is a matter of considerable importance to know, before selecting an inert solvent for extraction purposes, whether it boils at 35° or 100°. Whether some modification of fat enters into the structure and composition of all bacterial cells has not been established, but it is true that in the case of tubercle bacillus this organic constituent is a very important one and somewhat troublesome from an analytical point of view.

One of the most difficult and important problems that confront us in our analytical work to-day on tubercle bacillus is that of interference of the fat and waxes with the extraction and separation of the protein principles of the cell. This question will probably present itself in every future analytical research dealing with a determination of the composition of bacterial cells. In tubercle bacilli these fatty constituents represent a very large proportion of the cell and offer a resistance against the invasion of water into the cell structure. They are powerful obstacles to chemical extraction. It is necessary to overcome this by removal of this fatty constituent without alteration of the character of the protein principles functioning in the cell. In order to accomplish this, we must proceed under conditions that will not lead to coagulation of albumin. In order to overcome this barrier, we have adopted the principle in our work of "ether plasmolysis," which was applied so successfully by Chibnall in the case of plant cells from alfalfa and spinach. Treatment of desiccated tubercle bacilli for prolonged periods at ordinary temperature with ether serves not only to remove all ether-soluble fat and free fatty acids, but the treatment also alters the nature of the protective membrane entering into the structure of the cell, and thereby favors the penetration of water. This procedure leads to the practical result that, by cold-water extraction after ether plasmolysis, a water-soluble protein can be separated representing about 6 per cent of the original weight of the desiccated cell. If, however, the tubercle bacilli cells are autoclaved before "ether plasmolysis," practically no water-soluble protein can be extracted. From the preliminary reports which have been submitted to us by workers in the biological field it is quite apparent that this particular protein fraction is

the most potent tuberculin preparation that we have thus far succeeded in separating from the desiccated cell. In other words, we must operate at low temperature, and if we change our technique by removing fats and waxes, for example, by digesting with boiling toluene or boiling alcohol, we alter the character of certain active protein constituents, thereby making them insoluble in water and weakening their potency as tuberculin preparations.

Before we are in any position to discuss quantity production of tuberculin preparations from desiccated tubercle bacilli it is absolutely necessary to ascertain the relative value of the different protein fractions separated under the conditions of analytical procedure adopted. As I have stated above, it is possible to separate by cold-water extraction a protein fraction equivalent to 6 per cent of the weight of the dry cell. Doctor Long reports for this protein a very high tuberculin activity. If, therefore, we decided to adopt this fraction as our tuberculin preparation and to disregard the remainder of our cell, we would have a basis of calculating the cost of a definite product of commercial value. But other considerations enter in here which must be taken into account. We find that the fraction remaining behind after removal of this water-soluble protein fraction still retains strong tuberculin activity, but much less than that of the fraction soluble in cold water. This protein insoluble in cold water can be extracted with dilute alkali and be purified by precipitation with acids, and it still retains a very pronounced potency as a tuberculin preparation. Are we to throw this fraction away as useless material, or are we to appropriate it in our adoption of a standard tuberculin unit? It is very important in the light of these results to come to a very definite conclusion as to what shall constitute a standard tuberculin unit. Will we adopt one more active than that represented by our first water-soluble fraction, or will we choose a weaker unit and by dilution utilize a large part of the cell protein which, under the first experiment, will be thrown away? In other words, in my opinion, all of these questions must be very carefully considered before we can come to a definite or final conclusion regarding any recommendation for standardization of our manufactured product. If we can conserve in anyway active material of the cell which, according to present practice, is now thrown away in the process of manufacture, it is very important that we do so. It is undoubtedly true that we have before us the possibility of developing and utilizing a larger proportion of our desiccated tubercle bacillus cell than has hitherto been possible by present-day methods of commercial manufacture.

In all work dealing with the preparation of protein principles from bacterial cells it will be necessary hereafter to consider very carefully

the question of the action of alkali on these products. The protein tuberculin fractions are very sensitive to the action of alkali, and protein extracts which are very potent as tuberculins are weakened in their activity by alkali treatment. What change takes place in the protein molecule by action of alkali it is difficult to say at this date, but it is very probably associated with an alteration in the amide or polypeptide structure of the protein molecule.

In the carrying out of a line of research such as is represented by our investigation of the chemistry of the tubercle bacillus, and directed toward a search for that respective fraction which will exhibit the highest tuberculin activity, it is necessary that the chemist have the closest cooperation of workers trained in certain fields of biological research. The development of any method for the separation and purification of an active tuberculin fraction can not be accomplished successfully without the assistance of experts who are competent to give accurate reports regarding allergic, serological, and cytological reactions of the various fractions which we succeed in separating. Unless such tests are made for us during the actual course of our chemical investigation, we are severely handicapped in the progress of our work, and no means are available whereby we can decide whether we are working in the right direction. It is necessary to have beacon lights to guide us in our course, otherwise we are likely to waste valuable time in unprofitable work.

In our researches during the past two years we have had the good fortune of having associated with us in the direction of our joint research, Doctor White, who has perfected an organization which has served successfully to promote the preliminary development of our major problem. We are fortunate in having his hearty cooperation and interest. The preliminary tests on allergic reactions which have been made for us by Doctor Long have been extremely helpful, and have served many times to steer us along the road which has led to progress in our work. We have been awaiting the reports of Doctor Pinner and Doctor Cunningham with very much interest. There is no doubt in my mind that the continuation of our plan of close cooperation is bound to lead to the discovery of new facts and the contribution of valuable data which will aid in the solution of the tuberculin question.

## GENERAL DISCUSSION

Surgeon General CUMMING. Doctor Smith, will you open the general discussion on these papers?

Doctor SMITH. The question of tuberculin is evidently an important question, judging from the papers to which we have listened to-day. My personal experience rests with the old Koch period when we worked with Old Tuberculin. In the years preceding 1913

I spent a great deal of time on this subject from the serological point of view, but much of the work was never published because it never reached any stage of completion.

There are still a good many questions in my mind which make me wonder whether the study of the ordinary tuberculin has been carried to a satisfactory point as a preliminary for this work, and I was very much interested in what Doctor Johnson said about the difference between the boiled tubercle bacillus and that treated in the cold. I remember distinctly the great allergic reaction of the ordinary filtrates and the cultures in those years, but I remember that the cold filtrate was much stronger than the boiled. I remember distinctly tuberculin reactions in which 0.1 cubic centimeter would prove fatal to a tuberculous guinea pig. I know that some tuberculins would be only one-third of this strength.

Now, it seems to me that we have not entirely exhausted that preliminary study which will enable the chemist to know what is the most satisfactory substance for him to work with—what are the actual chemical foundations of tuberculin. It seems to me that the aim of all those who are working with tuberculin is to determine what that substance is. We have just listened to Doctor Pinner's paper in which he showed that there is a distinct difference between the complement-fixation, precipitin, and allergic fractions. What we want is knowledge of the allergic fraction; this is what we depend upon. We know that the allergic reaction is a safe test on which to base a diagnosis in a very high percentage of tuberculous animals—at least in 95 to 99 per cent. It is of extreme interest to know that serological tests are not of themselves adequate and we must after all get back to the live tissue of the animal body in order to get that reaction which will be most useful in practical application to man and animals. There are a great many unexplained questions in these fields which, I presume, will be summarized by those in charge of this work.

There is one question I wanted to ask: Has anyone studied comparatively the very old prolonged cultures which have been growing for many years and those which have been isolated relatively recently as regards lipoids? It seems to me that is something the biologists could do for the chemists, to show the difference between the 20-year-old cultures and the 18-months-old cultures and the lipoid ingredients. Possibly this has been done and is on record.

Surgeon General CUMMING. Doctor Krause, will you continue the general discussion?

Doctor KRAUSE. What we have heard here to-day confirms much work that has been done in the past. Personally, I can not see any other approach to the standardization of tuberculin than the one that stands out prominently in these papers—that is, the application

of the allergic reaction. Most past work has shown pretty definitely that it is the protein fraction that elicits the allergic reactions, but I also think that if lipin were added to the protein one should get much stronger reactions. For this reason I was wondering whether, if Doctor Pinner had added a little lipin to the fraction which he got from Doctor Johnson, the action of his antigen would not have been enhanced. This might very easily be studied along the lines of Doctor Pinner's work.

I was greatly impressed by the general trend of Doctor Long's remarks, and I wondered whether animals might not vary in giving testicular reactions as they do in their skin reactions. I should like to hear this discussed a little more in detail. In using the allergic reactions as a method of standardization I agree thoroughly with what Doctor Schroeder has said that "tuberculin will stand a tremendous amount of manhandling." It is certainly true that complement fixation and other serological methods do not offer any hope in regard to the standardization of tuberculin, because, as Doctor Long said, we understand by tuberculin only a something which gives a definite effect, and it seems to me that we must work on that effect—that is, the allergic response to the tuberculin—if we are going to standardize tuberculin.

Surgeon General CUMMING. Doctor Hektoen will you continue the general discussion?

Doctor HEKTOEN. I have not anything to say in addition to what has been said by previous speakers, but I should like to voice the impression I have obtained from what has been said—namely, that it seems to me the stage is clearly set for continued active cooperative work on questions concerning tuberculin and its standardization. I can not say anything in the way of definite details, but the impression is made very strongly that this work should be carried forward actively.

Surgeon General CUMMING. Doctor Lewis will you continue the general discussion?

Doctor LEWIS. It seems to be generally agreed here that the complement fixation and precipitation reactions are not adequate for the determination of tuberculin potency. The two reactions first mentioned may perhaps be closely interrelated, but they certainly rest on a different basis than the allergic reaction, when the chemical nature of the respective antigens is considered, and can not be taken to represent it actually in however many cases the relative potencies may roughly coincide, and it is well that we seem to be able to reach this conclusion.

Until a few months ago at least tubercle bacilli were frequently spoken of as "defatted" when they had merely been kept for some

time in cold alcohol. To speak of the bacilli as "defatted," if by that we are to understand that the fats and lipoids have been completely removed, is absurd when such a limited extraction has been used and is of difficult and uncertain accomplishment when even the most vigorous and thorough extraction methods are employed. The term has been so loosely used so long that it would seem advantageous to eliminate it entirely. Certainly no work to-day can be regarded as informing unless the exact extractive measures used are described and their results stated in terms of the amount of extractive still retained by the residue.

With regard to intracutaneous methods of testing the potency of tuberculin on guinea pigs, I should like to remark that Roemer is responsible for the idea that such a method could be worked out. We have never considered that our work (Lewis and Aronson) furnished a complete background for a system of testing, but thought when it was done, and I still think, that the interest in the idea should be kept alive.

In this connection I should like to inquire of those present who have the duty of producing and testing tuberculin whether there are essential difficulties in the practice of the older methods, and, if so, what these may be. Is there really difficulty in determining the potency of a recent preparation compared with one kept in the laboratory as a provisional standard? Is there real difficulty in turning out a product which will compare favorably in potency with an acceptable standard. From the standpoint of the experimental laboratory it has apparently been assumed for many years that these processes could be carried out in practice with relative ease and certainty. Is this assumption justified?

I would only say further that if it were considered desirable to substitute an intracutaneous for a subcutaneous tuberculin test as either a provisional or final step in determining tuberculin potency, we should not limit our vision to the guinea pig. An animal such as the calf, presenting a much less limited skin area, might prove more useful in practice.

Surgeon General CUMMING. Doctor Williams, will you go on with the general discussion?

Doctor WILLIAMS. On the scientific discussion I do not feel able to comment at all, but I should like to say that it is a source of great satisfaction to me and to the National Tuberculosis Association to have an opportunity to see the close cooperation that is being carried on in this work between the Bureau of Animal Industry and your own bureau; I feel that the correct thing is to seek further knowledge in this field, and I am particularly glad to have heard Doctor Hektoen's statement in regard to the cooperation between the different workers

who are carrying on research in association with the National Tuberculosis Association. Three or four years ago I felt that it might be very difficult to get that cooperative effort, but we seem to have been able to obtain it through the Research Committee, and perhaps that committee has been more or less instrumental in bringing about the cooperation between the three different institutions here to-day. I think you know, Doctor Cumming, that I have always been much interested in the relation between voluntary and official agencies and have tried to see that the official agency had and kept its proper place, and we want to keep our proper place and not overstep on the ground of the Federal agencies. It is this very efficient cooperation which I am delighted to witness.

Surgeon General CUMMING. Doctor Dorset, will you close the general discussion?

Doctor DORSET. The standardization of tuberculin differs for human and bovine practice. The extreme accuracy required for the former is not quite so necessary for the latter. At the same time tuberculins now on the market in bovine practice vary considerably; some are superpotent and some are at times so weak as to be useless. Even the product of one manufacturer may be superpotent one year and the next year almost devoid of potency. It is difficult to explain these occurrences, but I have thought it is because manufacturers do not give proper attention to the essential factors in production. If something could be done in the way of standardization of production, it would help to provide a standardized product for veterinary purposes.

We do not test every batch of tuberculin that we produce, but we have standardized our methods and we insist that they be always uniform. Now, what has been the result? This tuberculin of ours, while probably not so potent as that which may be found on the market at times, according to the results of Doctor Schroeder's test over a period of a good many years, tested out a good many times a year, runs very uniform on guinea pigs; it will kill a tuberculous guinea pig in a certain dose, and we can expect it to do that every time. Therefore, we feel that we have a sufficiently standardized tuberculin for veterinary use; and this has been accomplished by standardized methods of production. Thus I am interested in the second question that was submitted—that is, the control of standardization and methods of production.

I think you may all easily see by looking at these compilations which Doctor White has prepared from the data secured from 13 manufacturing firms, that there is a wide variation in the methods of production in commerical practice. We have found that our mass of bacilli after filtering is 0.5 gram per liter. I have thought sometimes of using this as a criterion of the amount of tuberculin that

should be produced. As to the strains of bacilli, ours came from Doctor Trudeau about 1891. We have the same culture growing in our laboratory now. Doctor Schroeder got his from Saranac Lake about 1891. I have recently been injecting a series of animals with some tuberculins from the market, comparing them with our regular tuberculin.

(The meeting adjourned for luncheon at 12.45 o'clock p. m.)

## SPECIFIC DISCUSSION AND CONCLUSIONS

(Afternoon session)

Surgeon General CUMMING. Doctor White, will you comment on the analysis of the League of Nations' questionnaire?

Doctor WHITE. There is before each one of you a copy of the questionnaire that was sent by the Health Section of the League of Nations to the Surgeon General to distribute among the different manufacturers of tuberculin in the United States and a synopsis of the answers. It will be apparent from the answers to this questionnaire—

*First.* That the United States Bureau of Animal Industry produces a large part of the tuberculin used in the United States—that is, 15,974,500 doses as against 5,469,522 doses. Thus the Bureau of Animal Industry produces nearly three times as much tuberculin wholly for animal diagnosis as all other manufacturers combined.

There has been a steady decline in the production of tuberculin for human use in the past three years and an increase in that used for diagnosis in pigs and chickens coincident with the apparent increase of this disease amongst these animals.

*Second.* There is no uniformity as to culture medium, strain of organism, period of growth, after treatment—that is, sterilization, filtration, dilution, or use of preservative.

*Third.* In only two States is there a law covering standardization.

*Fourth.* Where tests of strength are made there is no uniform method of infecting the guinea pigs used, nor of determining the infecting dose which they receive.

Altogether the procedure in manufacture is variable and much can be gained by uniform practice. As Doctor Dorset has shown, a uniform practice in production yields a very uniform strength of tuberculin year after year.

The two bureaus responsible for licensnig the manufacture of tuberculin are a part of this conference, and I am sure manufacturers will welcome any suggestions from them which will improve their product.

Suggestions as to strain of organism, medium, size of flask, surface area to bulk of medium, time of growth, weight of growth, after

treatment, preservative, dilution, and testing of strength would mean great improvement, even though the bulk of production is largely confined to one laboratory.

I think now, sir, with this material and the morning papers the conference may proceed to a discussion of the individual questions.

Surgeon General CUMMING. We have offered for your discussion three questions:

*First.* Have we sufficient knowledge at the present time to warrant the establishment of a standard for this substance?

*Second.* Can we, with our present knowledge, make important improvements in the present methods of manufacture of tuberculin and What should these improvements be?

*Third.* What lines of study are the best for us to pursue to enable us to arrive at a better standard of tuberculin?

We will now present the first question.

Doctor SMITH. In order to bring the question before the conference, I make the following motion:

> We have not at present sufficient information to recommend definite changes in the standardization and testing of tuberculin as now practiced by different countries, nor shall we have until the different methods proposed have been exhaustively studied comparatively.

Doctor HEKTOEN. I will second this motion.

Surgeon General CUMMING. The motion is now before you for discussion.

Doctor DORSET. As I understand the motion there is not sufficient knowledge available to us at the present time to warrant the adoption of an international standard. Because we do, I think, find it necessary to have some sort of test for tuberculin, some method that we call "standardization" but which is not exact enough to warrant its establishment as an international guide. But we need some sort of guide. I am sure that the conference does not intend to take the position that there is no means by which the relative potency of tuberculins can be determined at the present time, or to take the position that such a thing could not be done in a rough way. But we should not make this international.

Dr. SMITH. The motion is intended to imply that we are not prepared to disturb the present methods.

Before we have a decision may we have a statement as to present methods from Doctor Dorset? We probably all know them, but it might be well for Doctor Dorset to state them as practiced by the Bureau of Animal Industry in this country.

Doctor DORSET. As far as our own practice is concerned, we do not test the potency of each batch of tuberculin as it is prepared. We specify the method of production, and batches are tested at intervals. Doctor Schroeder tests these by the guinea-pig-killing test—that is,

the lethal dose of the tuberculin for tuberculosis guinea pigs is determined. This is really our method of standardization. The requirements for preparation are rigid and more constant than the actual testing of the product after it has been prepared.

Doctor STIMSON. We might divide the question into, first standardization of bovine tuberculin, which each state would determine for itself, prescribing the methods to be used in preparation with occasional control tests, and, second, the necessity for further comparative study to enable us to standardize human tuberculin with accuracy. Would that make our position clearer to those who did not hear the discussion but read the conclusions only.

Doctor LONG. It might be well if, in some way, this idea could be conveyed, Comparison of methods is necessary. Five or six methods have been proposed by various people, and to settle upon any one of these without a comparison would undoubtedly lead to error. There has been no adequate comparison and there is no reason for upsetting present practice until this is done. Picking out one method without comparing it is not exact.

Supposing that an international standard were adopted by the League of Nations committee, how far would that have weight or force? Would it be merely a focal point about which methods would eventually be expected to crystallize or would it have weight in many places even though it did not have here?

Surgeon General CUMMING. I think it would have a good deal of weight in European countries and in South America. In England they are going through the throes of standardization, are they not Doctor White?

Doctor WHITE. They are having the same discussion in England as we are having here. The Medical Research Council has just published a report of their results of comparative tests of tuberculin in cattle (Special Report Series No. 94). Among the questions facing the English committee was one, "Can the present methods of standardizing tuberculin be improved?" A method of standardizing Old Tuberculin by a precipitin method is offered by Professor Dreyer and Mr. Vollum; but, as you have seen, using pure fractions, there is no definite relation between the precipitin fraction of the bacillus and the allergic fraction. So far as the testing of cattle allergy is concerned, the English committee recommends a double intradermal injection on the same skin area of the neck of the animal to be tested.

In Canada, Doctor Watson and Doctor Heath suggest a complement-fixation method which runs roughly parallel with Doctor Schroeder's tests. But here again it would seem that allergy fractions and antigenic fractions for complement of the tubercle bacillus are not the same.

This conference, therefore, must look toward a plan for separating these different fractions and isolating the allergic fraction in stable form which can be readily diluted as the first step in standardizing tuberculin.

Doctor SCHROEDER. I sent Watson a dozen or so samples of tuberculin I had tested. These he tested by the complement-fixation method and reported results to me. While the results he obtained were not like mine, they were sufficiently like mine by the reading that, if I had based reactions on these tests instead of on my own, they would have been practically the same.

Surgeon General CUMMING. The question now before us, moved by Doctor Smith and seconded by Doctor Hektoen, is:

> We have not at present sufficient information to recommend definite changes in the standardization and testing of tuberculin as now practiced by different countries, nor, shall we have until the different methods proposed have been exhaustively studied comparatively.

The conference unanimously adopted this resolution.

Surgeon General CUMMING. We now come to the second question: "Can we, with our present knowledge, make important improvements in the present methods of manufacture of tuberculin and, if so, what should these improvements be?"

Doctor WHITE. In presenting that question it will be clear, by referring to the answers to the questionnaire, how enormous is the production of the Bureau of Animal Industry as compared with all the rest of the United States. There are only two States that have a law covering any test of tuberculin, and neither the Bureau of Animal Industry nor the Public Health Service has suggested, up to the present time, to the different manufacturers how they should proceed save when they have been asked for advice.

Doctor DORSET. The Bureau of Animal Industry has made certain requirements of producers of tuberculin. For example, we have required that their culture media shall be placed in containers so that the surface area of the medium in the container shall bear a certain relation to the total volume of culture fluid. I forget the exact requirements, but I think it is about 8 square inches of surface to 100 cubic centimeters of broth, and the manufacturers are required to put their medium in containers in this proportion. We found one commercial producer making tuberculin in large flasks holding about 3,000 cubic centimeters with a surface area equivalent to that required for 100 cubic centimeters of broth, in other words a tremendous amount of culture fluid and very minute surface area. And the the tuberculin from this producer apparently led to some disastrous results in some herds on which it was used.

The relation of the amount of growth to the total amount of culture fluid must be taken into account, and we require this also. I

do not believe that we can go any further except to say that true tubercle bacilli must be used. We require that cultures be submitted to our laboratories and they are always examined before a license is issued.

Doctor WHITE. It seems to me that the Bureau of Animal Industry and the Public Health Service should have standards of methods for all producers of tuberculin.

Doctor DORSET. Probably one thing that has prevented this is the feeling that perhaps others could make as good tuberculin as ours by different methods; but the nearer we can reach a standardization the better.

Doctor STIMSON. If the Bureau of Animal Industry does not control absolutely the tuberculin for bovine use, the Public Health Service does still less in the way of applying anything like standardized methods for the production of tuberculins or substances which would have to be handled under that term for human use. These substances are produced by so many different methods, some of them growing the tubercle bacilli on all sorts of media and handled in all sorts of ways, that we can not possibly get at a method which would cover them all, unless we arbitrarily make a standard for the production of therapeutic tuberculin for human use and refuse to license any other kind. That would put us in a position of depriving members of the medical profession of preparations which they believe to be of use. If we believed that the preparations they want are no good and had good reason to back up our belief, we could do it. Considerations like this are what led me to suggest in the beginning that we might have to split this whole question into tuberculin used for diagnosis of tuberculosis in cattle, and tuberculin, or substances which would have to be included under the generic term of tuberculin, to be used for diagnostic and therapeutic use in human beings. Doctor McCoy is more familiar with the details of the administration of the biologic act as it refers to tuberculin, and I would like to hear what he thinks about the possibility of standardizing tuberculins for human use.

Doctor MCCOY. I have very little to add to what Doctor Stimson has said. One of the reasons why we never pressed the matter of standardization at the Hygienic Laboratory, in addition to the reasons given by Doctor Stimson, is the very fact that 99 per cent of all that is produced is used for diagnostic purposes and comparatively little for treatment. Our law covers only prevention and treatment. It does not cover diagnostic agents.

We have had so many other matters that appeared to us more pressing problems of standardization and to afford better prospects of solution that we have ignored tuberculin. I do not recall, how-

ever, a meeting of our advisory board when the matter has not come up.

Doctor WHITE. You have no jurisdiction in diagnostic uses?

Doctor McCOY. No. The law covers only prevention and treatment. If there appeared to be a reliable and satisfactory method of standardization we could, of course, enforce it; but, as Doctor Stimson said, there are so many tuberculins actually in use to meet the preferences of individual groups of physicians that standardization seems extremely difficult.

Doctor DORSET. The law that the Bureau of Animal Industry works under is worded almost identically with yours. It applies to any virus, serum, toxin, or analogous product used for the treatment of domestic animals. If your interpretation of the word "treatment" is correct, I suppose we have been exercising illegal control over the production of tuberculin in cattle. We felt that the word "treatment" meant *use* on the animal, not necessarily therapeutic use, and we have so considered it.

Doctor McCOY. Our law reads for the prevention and cure of diseases in man.

Doctor SMITH. Should you not argue that diagnosis is a necessary preliminary to prevention and treatment?

Doctor McCOY. We have stretched it a little, and manufacturers have asked that we stretch it to cover such a diagnostic agent as that used in the Schick test, which is also used as preliminary to active immunization.

The producers are always anxious that we protect them.

Doctor SMITH. Can you treat without diagnosis?

Doctor McCOY. I feel that, with our influence with the producing houses, we would have no difficulty if we were to attempt to establish a standardized tuberculin. We have had a decision on the copy of the law as applied only to prevention and treatment, not to diagnosis. If it is applied to diagnostic agents, it would lead us very far afield, and I do not know how we would control it.

Doctor SMITH. It seems clear that the strength of tuberculin should be the same throughout the United States. When tuberculosis has become less and less, there will be growing up a susceptible stock and there must be a diagnostic agent which is above criticism. Because of the growing importance of tuberculin in diagnosis in disclosing tuberculosis as a disappearing disease, it becomes a question of economics and we must take cognizance of it.

Doctor WHITE. There will undoubtedly arise within a year or two the question of production of tuberculin on synthetic media. Already, under Doctor Dorset and the Research Committee, work has progressed very rapidly. It seems fairly certain that the tuberculin substance of the tubercle bacillus should be extracted in the

cold; that heat coagulates a large part of the active substance which then is precipitated; and that the bacillus contains a very large fraction easily extractable which is now wasted.

These considerations, if proved, justify the action taken on the first question of the day; but as they are not yet exact enough for practical use, a definite prescription for the manufacture of Old Tuberculin, following the practice of the Bureau of Animal Industry, would be very welcome and would form a base line from which future uniformity would naturally follow as knowledge grows.

Doctor DORSET. The term "tuberculin" is so broad that you can not possibly get a set of regulations to cover tuberculin in general, but I believe the term "Koch's Old Tuberculin" means a fairly definite thing; it means the organism cultured on a meat broth with peptone added. I do not know whether or not you could restrict that to the human type of bacilli. I do not remember whether Doctor Koch used veal broth or beef broth, but Koch's Old Tuberculin is the chief tuberculin used, and if that were defined it would leave everybody free to prepare other tuberculins, but they could not use the name "Koch's Old Tuberculin" without conforming to certain specifications. If it were decided by an authorative body that Koch's Old Tuberculin meant a certain thing, we could see that that was used.

We have not felt that we should restrict the methods that are used in producing tuberculin. We have ourselves been using experimentally some 40,000 or 50,000 doses of synthetic tuberculin. In preliminary tests this appears to be superior to that which we have been getting from broth cultures. If we had been restricted only to broth cultures, then we might have excluded experimental work and thus have stood in the way of improvements in the industry.

The nearer we can reach a standardization the better. I am glad to hear Docter Smith's suggestion. We have gone about as far as we dared go. If we ought to go further, I am sure we would like to.

Doctor SMITH. Would not the synthetic tuberculin be cheaper?

Doctor DORSET. It will not be more expensive. Although asparagine is very expensive and would cost above what is spent for meat, I believe there would be considerable saving of labor and we might be able to produce it cheaper.

Doctor SMITH. Are you likely to find that there are certain reactions that are absent in the synthetic medium?

Doctor DORSET. We expected that we would do away with certain reactions; but instead of that we have more positive reactions from the synthetic cultures than from the broth cultures, thereby indicating that there was nothing in the broth that was causing an undue number of reactions. The difference between the two appears to be that the synthetic tuberculin reacts when the ordinary broth tuberculin does not, and we do not know just why that is.

The manner of growth on the two media is different. On broth the *p*H reaction of the medium with human bacillus rises and then falls quite sharply. On the synthetic medium we do not get this curve; it rises sharply and continues on a plane similar to that of the bovine bacillus. I do not think we should say that cultures three or four weeks old are old enough to produce tuberculin. The period of standing may be an advantage. With the synthetic medium the growth continues apparently over a much longer period of time.

Doctor LEWIS. I would propose the following motion:

The commission believes that a test based upon the following definitions will aid in bringing about a uniformity in practice:

Tuberculin O. T. should be defined as a product derived from bouillon cultures of the tubercle bacillus (human type) by filtration and concentration and should have the following essential characteristics:

(*a*) It should cause typical symptoms of allergy (constitutional or skin reactions) in tuberculous animals and at the same time be without effect on normal animals.

(*b*) Its potency should be sufficient to cause the death of tuberculous guinea pigs within 24 hours after intraperitoneal injection of 0.25 gram doses per 500 grams of guinea pig weight.

In practice the strength of other forms of tuberculin should be computed in terms of O. T. (Old Tuberculin).

Tuberculin from bovine strains of tubercle bacilli should conform to the standard for O. T. (Old Tuberculin).

Tuberculins from avian strains of tubercle bacilli do not conform to this standard for O. T. and should be considered independently.

It is desirable for Government bureaus concerned with licensing tuberculin to issue a statement of a method with which a product of suitable potency and purity is more or less uniformly obtainable.

Doctor DORSET. I second the motion.

Surgeon General CUMMING. You have heard the motion; what is your pleasure?

Doctor SMITH. Before voting I would like to ask if there are any data to determine whether abundant growth of bacilli means greater quantity of tuberculin and whether more tuberculin is derived from bacilli from say 18 to 20 years than from recent cultures of say 1 to 2 years on artificial medium.

Doctor DORSET. I think that with a given culture the amount of growth is a pretty good index of the tuberculin produced. The tuberculin arises only from the growth of the bacilli. The more bacilli, the more of the active substance that is extracted by the culture medium up to the limit of solubility. If we could control tuberculin, one of the first steps would be to require a certain weight of tubercle bacilli per gram of Koch's Old Tuberculin.

On the second question I have no data.

I would not propose that weight alone be a guide. Even though it is true that the tubercle bacilli are extracted slowly, that could be taken care of by prescribing a certain period during which the cultures

must remain in the incubators, and when the product is finished and the bacilli are filtered off, there must be a certain weight of bacilli as representing a certain amount of tuberculin.

Doctor LONG. Would that not be an important point to add to your specification, that a certain amount of time be allowed to elapse before maturity is reached?

Doctor DORSET. One culture may be inoculated and grow only half as rapidly. The one growing more rapidly and producing more material, if left in the incubator the same length of time as the other, would have the greater strength.

Doctor PINNER. Is there any evidence that uniformity of production would insure a uniformity of potency? If we had uniform directions for production, we would not be any more sure of potency.

Doctor DORSET. To reply to Doctor Pinner, I understand him to ask whether there is any evidence that uniformity in methods of production will necessarily bring about uniformity of potency. I think I referred this morning to the fact that we have had uniform methods of production for a long time with our product and that it has been submitted to repeated tests by Doctor Schroeder and that it has always shown a remarkable uniformity in potency, therefore indicating that uniform methods of production do result in production of tuberculin of uniform potency.

Doctor PINNER. Were the strains uniform?

Doctor DORSET. Yes; had we changed our strains we would have had different results, but I take it that uniformity of methods of production includes uniformity of strains of tubercle bacilli used for the purpose proposed.

Doctor KRAUSE. What about foreign tuberculins?

Doctor WHITE. Foreign tuberculins are more easily handled under the same jurisdiction. If the methods of preparation were uniform, it would wipe out a good many of the gross differences that exist to-day and would regulate foreign tuberculins in the same way as domestic. Biologic products crossing Federal or State borders are much easier to control than those within State lines.

Doctor MCCOY. The restrictions are the same. So far as human tuberculins are concerned, the question is merely academic. We do not have two importations a year of foreign biological products. If we decided that it was wise to prescribe that a certain human tubercle bacillus culture be used, we could insist on the foreign firms doing the same as the American. How to detect infraction of the regulation is a more difficult question. Unless it becomes an article of interstate commerce, we have no control.

Surgeon General CUMMING. Are you ready for the question?

(On vote the resolution proposed by Doctor Lewis, seconded by Doctor Dorset, was unanimously adopted.)

Surgeon General CUMMING. The third question before you is, "What lines of study are the best to enable us to arrive at a better standardization of tuberculin?" Doctor White, will you outline the present plan of study?

Doctor WHITE. The present plan of study is one of cooperation between the Hygienic Laboratory of the United States Public Health Service, the Bureau of Animal Industry of the Department of Agriculture, the Research Committee of the National Tuberculosis Association, and the University Research Laboratories. The chairman of the Research Committee is in charge of the division of tuberculosis research in the Hygienic Laboratory, thus making a Government bureau the clearing house for the knowledge accruing from the different researches.

The part of the work directly bearing on the subject of tuberculin is carried on as follows:

Large quantities of tubercle bacilli have been prepared on a synthetic medium as follows:

*Formula.*—All ingredients for this whole work were purchased at one time and were of known composition. This was true also of the glassware. Parke, Davis & Co. and H. K. Mulford & Co. have cooperated in doing all the work of growing the bacilli. When each crop is ready, it is carefully filtered; the filtrate is sent to Doctor Long for analysis, and the bacilli, air-dried, are sent to Doctor Johnson at Yale.

A careful plan of chemical fractionation is followed on both of these primary divisions of the growth of the bacillus. The bacilli are presumably in large measure living at the beginning of this work. As each fraction is separated, it is sent to the chairman of the Research Committee and by him is distributed to different laboratories for serological, cytological, and allergic studies. The results of these studies are brought together from time to time in conference, and the knowledge is correlated, and the next steps in analysis are decided upon. The different workers are not familiar with the steps taken by the chemists, so that they are not influenced in their interpretation by a knowledge of the substance with which they are dealing.

The conferences for correlation are held before a small jury of experts carefully chosen for criticism and advice. Already it is quite clear that different fractions are concerned with different phenomena, and it seems promising that this group action will rapidly advance our knowledge so that soon we may be able accurately to deal with this problem. The chance for field study of fractions is opened by the cooperation of the Bureau of Animal Industry.

This is only one of the departments of the work of the committee. The others deal with the anatomy of the lungs, the biology of the tubercle bacillus, the cytology of animals suffering from infection with tuberculosis, the development of fibrous tissue, and the relation of primary to secondary infections. While the details of these studies do not belong here, they are all of help in putting together the puzzle of this disease.

I shall be glad to answer any questions.

Doctor DORSET. I move that—

This commission approves the present cooperative plan of research being carried on by the Research Committee of the National Tuberculosis Association in cooperatiou with the Hygenic Laboratory of the United States Public Health Service and the Bureau of Animal Industry as the most likely procedure to bring about a better understanding of tuberculin and its action and enable us to arrive at better methods of use and standardization, and the commission urges that this work be continued.

Doctor HEKTOEN. I second this motion.

Surgeon General CUMMING. Any discussion?

(The motion was unanimously adopted.)

Surgeon General CUMMING. I wish to express my appreciation to you gentlemen for having come here and for doing all the work you have done. I am sure Doctor Mohler and the National Tuberculosis Association join me in this.

(The meeting adjourned at 4.45 o'clock p. m.)

## APPENDIX A

Adresse télégraphique Nations Genève.

SOCIÉTÉ DES NATIONS. LEAGUE OF NATIONS.

GENEVA

The Health Committee of the League of Nations has been engaged for several years in the study of the standardization of sera, serological reactions, and biological products. The task of carrying out these researches has been entrusted to a group of European and American experts. Results of international importance have already been reached, in so far as the unification of methods to be applied to the titration of several sera and biological products is concerned.

The Health Committee decided at its last session to take up the study of the standardization of tuberculin and appointed a special commission under the chairmanship of Professor Calmette of the Pasteur Institute to examine this problem.

This commission has come to the conclusion that before submitting proposals concerning the standardization of methods it will be necessary to obtain as complete information as possible on the processes of preparation and of titration employed by the various institutes producing tuberculin. For this purpose the commission has decided to apply to all institutions issuing tuberculin for medical or veterinary use—both official and private—requesting them to fill up a questionnaire concerning their methods of preparation and titration of tuberculin.

A copy of the questionnaire is inclosed herewith, and we should be grateful if you would kindly complete it and return it to us at your earliest convenience.

HEALTH SECTION.

## APPENDIX B

### LEAGUE OF NATIONS

HEALTH ORGANIZATION QUESTIONNAIRE REGARDING THE PREPARATION AND TITRATION OF TUBERCULIN

*A. Preparation of tuberculin*

1. What types of tubercle bacilli do you employ?
2. What is the composition of your medium?
3. What is the initial pH of your medium?
4. For how long is the culture incubated?
5. Is the culture killed before filtration?
   By heat?
   By some other process?
6. Is the volume of the culture reduced by evaporation before or after filtration?
7. Do you carry out filtration by:

| | | |
|---|---|---|
| Filter paper | No. | Mark. |
| Candle | No. | Mark. |

8. Do you sterilize the filtrate thus obtained
   By heat?
   By any other process?

9. Does the raw tuberculin you prepare represent a mixture of several tuberculins obtained from different types and strains of tubercle bacilli?

10. If such is the case, what are the proportions of human, bovine and avian tuberculins in the mixture?

11. Do you issue your tuberculin in diluted form?

12. If so, to what dilution of the raw tuberculin does it correspond?

13. Do you add to your prepared tuberculin any preservative substance? If so, what is the nature and proportion of the substance added?

14. (*a*) Do you make any preparations other than Koch's raw tuberculin?

(*b*) If so, please indicate, briefly, the process of their preparation, and their properties and characters.

(*c*) Are they destined for purposes of diagnosis, or for therapeutic uses?

(*d*) Do you prepare and issue special dilutions of raw tuberculin or of other tuberculins (which?) for the various cutaneous and intradermal reactions? In what form are these issued?

*B. Method of titration of tuberculin*

15. Do you make regular titrations of your tuberculin?

16. Is titration obligatory by law?

17. Do you make the titration on tuberculous guinea pigs?

18. What is the procedure followed and the type used in rendering the guinea pig tuberculous?

19. Do you make a preliminary test to determine if the guinea pigs are suitable for use in the definitive titrations?

20. By what route do you introduce into the tuberculous guinea pig the tuberculin which is being titrated?

21. Do you carry out titration as against a standard tuberculin, or do you simply determine the toxicity of your tuberculin by its effects on the guinea pig?

22. (*a*) From what laboratory does the standard tuberculin come?

(*b*) For how long have you used it?

(*c*) Do you keep it in liquid or in solid form?

(*d*) Is it stable?

(*e*) From what types has it been prepared?

23. Do you do a preliminary titration before the definitive one is carried out?

24. In carrying out the latter do you use one or several methods?

25. Is titration done in vitro? If so, is the process based on the principle of—

Complement deviation?

Or precipitation?

Or other biological reaction?

26. Is the titration of tuberculin carried out on the raw product, or after its dilution?

27. Is the tuberculin submitted to any other tests than those for determining its specific toxicity?

*C. Additional Questions*

28. Have you found any difference in the tuberculin produced by different strains of tubercle bacilli?

29. (*a*) If you use a single strain of tubercle bacillus what is its history?

(*b*) What is the character of its growth on different media?

30. What is the relation of surface area of culture medium to volume in each container?

31. What is the resulting weight of tubercle bacilli to 100 cubic centimeters of culture medium in the final separation?

32. If you sterilize by heat—

(*a*) What is the temperature used?

(*b*) What is the frequency of exposure to this temperature?

(*c*) What is the total length of time of exposure?

(*d*) What character of heat is used?

33. Has any attempt been made to standardize the guinea pigs used for testing the strength of tuberculin?

34. Has any attempt been made to standardize the tubercle bacillus with which you infect the pigs for testing?

35. How do you determine the dosage—

By counting?

Or by weight?

36. Is this dose given

Subcutaneously?

Or intraperitoneally?

37. If you have any suggestions to offer for the improvement of tuberculin manufacture, we shall be glad to have these suggestions inclosed on a separate sheet.

## APPENDIX C

### DATA SECURED FROM 13 FIRMS MANUFACTURING TUBERCULIN

(These firms were licensed either by the Bureau of Animal Industry or the Hygienic Laboratory)

| | |
|---|---|
| Number of firms licensed by Bureau of Animal Industry | 4 |
| Number of firms licensed by Hygienic Laboratory | 11 |
| Total | 15 |

Thirteen of these firms manufactured and distributed tuberculin as follows:

| Use | Production | | | | | |
|---|---|---|---|---|---|---|
| | Cubic centimeters | | | Individual doses | | |
| | 1922 | 1923 | 1924 | 1922 | 1923 | 1924 |
| Human diagnosis | 39,471 | 73,109 | 101,356 | 259,242 | 383,799 | 506,792 |
| Human therapeutic | 170,021 | 315,945 | 420,562 | 680,084 | 583,700 | 1,002,170 |
| Pig diagnosis | 1,691 | 9,168 | 11,358 | 6,764 | 36,673 | 45,435 |
| Bovine diagnosis | 1,010,816 | 723,368 | 681,796 | 4,043,268 | 2,893,596 | 5,420,786 |
| Chicken diagnosis | 502 | 841 | 825 | 2,011 | 3,367 | 3,301 |
| Unspecified | 24,134 | 34,521 | 30,508 | 96,535 | 138,045 | 122,056 |
| Total | 1,246,635 | 1,156,952 | 1,246,405 | 5,087,904 | 4,049,180 | 7,100,540 |
| Bureau of Animal Industry production | 2,977,800 | 2,639,750 | 3,121,600 | 8,036,500 | 12,084,237 | 15,974,500 |
| Total, United States | 4,224,435 | 3,796,702 | 4,368,005 | 13,124,404 | 16,133,417 | 23,075,040 |

## ANSWERS TO LEAGUE OF NATIONS HEALTH COMMITTEE

(NOTE.—Where the answers of the following do not appear for corresponding questions it is because no answers were given or were of no value.)

### *A. Preparation of tuberculin*

Answer 1. Of 13 firms manufacturing tuberculin—

6 use bovine bacilli for bovine tuberculin.
5 use human bacilli for human tuberculin.
3 use avian bacilli for avian tuberculin.
4 use mixed cultures (human and bovine strains are mixed in different proportions for human or bovine tuberculin).

Answer 2. For culture medium—

12 use glycerine (5 per cent in most instances, but ranging from 3 to 10 per cent).
5 use veal infusion.
5 use beef extract.
2 use beef infusion.
1 use Liebig's extract.
2 use peptic beef broth.
7 use peptone.
13 use salts. (These vary, usually potassium or sodium phosphate).

Answer 3. The pH of the medium varies from 6.8 to 7.6.

Answer 4. The incubation time varies from 1 week to over 3 months, averaging 8 to 10 weeks.

Answer 5. The cultures are all killed before filtration, by the majority with the Arnold sterilizer with a temperature ranging from 90° C. to 100° C., during one hour. One firm used 1.5 per cent phenol for two days.

Answer 6. The volume is reduced by evaporation—

After filteration by 7.
Before filteration by 3.
Intermittantly by 2.
(One firm reduces volume of human culture before filtration and the volume of bovine culture after filtration.)

Answer 7. The filtration method varies, the majority using paper and candle.

Paper and candle by 9.
Filter paper alone by 2.
Gauze and abs. cot. pads by 1.
Centrifuge and candle by 1.
Candle alone by 1.

Answer 8. The filtrate is sterilized by—

7 with heat.
4 with Berkfeldt.
1 with heat and filtration.
1 with phenol.
1 does sterility tests after filtration.
2 do not sterilize.

Answer 9. The raw tuberculin thus prepared comes in seven cases from unmixed strains and in six cases mixed strains, with no two proportions alike. Avian tuberculin, however, is usually from a single avian strain of bacillus.

Answers 11 and 12. Nine firms issue their tuberculin in diluted form and four issue it undiluted. The dilution varies from full concentration to eight one hundred thousandths of full strength with no uniformity save in those furnishing undiluted forms.

Answer 13. Nearly all these firms use a preservative, 0.5 per cent phenol being favored in most cases, but 3 out of 13 use none. There is, however, a distinction made between intradermic and subcutaneous tuberculin, no preservative being used with intradermic and ophthalmic tuberculin. There is no uniform practice.

Answer 14. Eleven firms make other tuberculins than Koch's, but use a modified form of Koch's in addition to the following variety of other forms:

1 water extract.
5 bouillon filtrate.
4 bacillus emulsion.
3 tuberculin residue.
3 special preparations for the eye (v. Pirquet and Mantoux).
1 from a specially prepared virulent tubercle bacillus strain for human therapy.

*B. Method of titration of tuberculin*

Answers 15, 16, 17. As regards the titration of tuberculin—

7 do not test toxicity.
6 test by guinea pig killing potency.

There is no uniformity in the method. All methods are modifications of the Bureau of Animal Industry plan. In two cases (Michigan and Missouri) State law was obligatory for the test.

Answers 21 and 22. One firm uses as a standard of tuberculin for comparison a sample from the Bureau of Animal Industry; one uses one from the Hygienic Laboratory, from which none is ever produced. The others use a standard of their own or none at all.

Answer 25. Neither complement deviation nor precipitation is used at all, although one firm uses skin tests for human tuberculin and one uses Schroeder's test with Bureau of Animal Industry standard.

Answer 26. Titration of tuberculin was carried out on the raw product in two cases and on the diluted and raw products in one case.

Answer 27. The tuberculin was not submitted to any test except, in seven cases, to a sterility test.

*C. Additional questions*

Answer 28. Three firms found a difference in the strength of the tuberculin when produced from different strains or tubercle bacilli; two did not find any difference; one found a difference in different crops of the same strain; and the others had not investigated.

Answer 29. The history of the strains of tubercle bacilli ranged widely, coming—

1 from Pennsylvania State Bureau of Animal Industry.
1 from Hygienic Laboratory.
4 from Bureau of Animal Industry.
1 from Nebraska Experiment Station.
1 from Saranac Lake.
1 from Doctor Ravenel.
1 from New York Department of Health.
1 from specially cultivated human strain.

The growth was carried on Dorset's medium, broth, or glycerin agar.

Answers 30 and 31. The relation of surface area of culture medium to volume in each container varies in each case, ranging from 10 square centimeters surface area to 100 cubic centimeters volume to 150 square centimeters surface area to 200 cubic centimeters volume. In the final separation the resulting weight of tubercle bacilli to 100 cubic centimeters of culture medium was not tested by 11 firms. By 2 firms the resulting weight was 5 grams per 100 cubic centimeters.

Answer 32. There is no uniformity in temperature or duration of sterilization. The Arnold sterilizer is used in most cases. The temperature ranges from 90° C. to 100° C., and the time of exposure from one hour, one exposure, to three hours—i. e., three exposures on successive days of one hour each.

Answers 33, 34, and 35. Six firms have attempted to standardize the guinea pigs used for testing by having the pigs of uniform weight and care. Nine attempts were made to standardize the dose of tubercle bacillus given to the guinea pigs used in testing the strength of the tuberculin—

in 4 cases by counting.
in 1 case by weight.
in 3 cases by volume.
in 1 case by loop.

Answer 36. The guinea pigs for this test are infected in six cases subcutaneously, in two cases intraperitoneally.

Answer 37. Only one firm suggests help. It suggests the development of growing tubercle bacilli on synthetic media.

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